

TABLE VII. Comparison of Clinicopathological Features of Patients With YMDD Mutants During and After 5-Year Treatment Period

Category	With YMDD mutation during 5-year (n = 230)	With YMDD mutation after 5-year (n = 21)	P-value
Age (years) ^a	44 (23–71)	50 (33–69)	0.109
Sex: male/female	194/36	14/7	0.063
Genotype: A/B/C/others	11/9/203/7	0/4/16/1	0.0184
Histology: no cirrhosis/cirrhosis ^b	185/43	19/2	0.384
Bilirubin (mg/dl) ^a	0.7 (0.2–16.5)	0.7 (0.4–4.4)	0.570
ALT (IU/L) ^a	118.5 (14–2,274)	142 (25–2,077)	0.527
GGTP (IU/L) ^a	58 (16–402)	82 (24–264)	0.382
Viral load (log copies/ml) ^a	7.2 (<2.7 to >7.6)	7.4 (<2.7 to >7.6)	0.936
HBeAg: positive/negative	128/102	7/14	0.0496

YMDD, tyrosine–methionine–aspartate–aspartate; ALT, alanine aminotransferase; GGTP, gamma glutamyltranspeptidase; HBV, hepatitis B virus; CHB, chronic hepatitis B; HBeAg, hepatitis B envelope antigen.

^aData are median (range) values.

^bChronic hepatitis and cirrhosis were confirmed by needle biopsy, peritoneoscopy, or clinically before treatment. Diagnosis of chronic hepatitis was based on elevated ALT levels over 6 months and absence of clinical evidence of portal hypertension, such as esophageal varices, ascites, hepatic encephalopathy, and imaging features suggestive of cirrhosis on ultrasonography. Viral load was measured by PCR. All viral loads below the lower limit of detection (<2.7 log copies) were set to 2 and those over upper limit of detection (>7.6) were set to 8 for calculation purposes.

of YMDD mutant in long-term lamivudine treatment than elderly patients.

Several new nucleos(t)ide analogs, for example, adefovir and entecavir, are available at present [Gish et al., 2007; Marcellin et al., 2008]. These new drugs have greater inhibitory effects on HBV replication and their use is associated with a lower incidence of drug resistance. However, resistant to the new drugs has already been reported [Suzuki et al., 2007; Baldick et al., 2008]. Lamivudine was the first nucleoside analog and has been used over a long time worldwide. Based on the result of our study, younger patients (<50 years) who continued lamivudine monotherapy without emergence of YMDD mutant during 5-year period showed less opportunity to develop mutants after a 5-year follow-up and were able to continue lamivudine monotherapy. After the cessation of lamivudine therapy, flare up of ALT accompanied with elevation of HBV DNA was observed at a high frequency [Song et al., 2000; Dienstag et al., 2003a; Akuta et al., 2005]. Moreover, we reported previously HBsAg clearance from the serum in some patients who received long-term lamivudine therapy [Kobayashi et al., 2007]. Taken together, it seems that before any treatment, one can predict a less likelihood of development of YMDD mutants during long-term lamivudine therapy in young patients with genotype C who are HBeAg negative, have no cirrhosis, and no elevated GGTP level. Tables II and VII suggest that patients with genotype B are also less likely to develop YMDD mutant, but their numbers are too small to make a firm conclusion. Further studies of larger number of patients with genotype B, A, and others are needed to clarify this issue.

In conclusion, factors associated with lack of appearance of YMDD mutants during 5-year lamivudine therapy in patients with HBV infection are HBeAg negativity, lack of cirrhosis, and high GGTP level. Patients who do not show the emergence of YMDD mutants during 5-year lamivudine therapy, younger age protected against the emergence of such mutants during the following 5 years of follow-up. On the other

hand, in those who show emergence of YMDD mutant, elevation of ALT or viral load correlate with a short latency to emergence of YMDD mutants, presence of mixed (YIDD + YVDD) type, and low baseline ALT level.

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Association of HLA-DR14 with the Treatment Response in Japanese Patients with Autoimmune Hepatitis

Yoshiyuki Suzuki · Kenji Ikeda · Miharuru Hirakawa · Yusuke Kawamura · Hiromi Yatsuji · Hitomi Sezaki · Tetsuya Hosaka · Norio Akuta · Masahiro Kobayashi · Fumitaka Suzuki · Satoshi Saitoh · Yasuji Arase · Mariko Kobayashi · Yuzo Miyakawa · Hiromitsu Kumada

Received: 30 December 2008 / Accepted: 10 August 2009 / Published online: 22 January 2010
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Abstract

Background Influence of human lymphocyte antigen (HLA) on the therapeutic response in autoimmune hepatitis (AIH) is not known.

Aims To evaluate if HLA-DR types influence biological and histological responses to corticosteroids in patients with AIH.

Methods During 28 years from 1979 through 2007, 48 patients with definite diagnosis of AIH received long-term corticosteroid therapy (median 9 years [range: 5–28 years]) in a single Japanese center. They were followed for transaminase levels and received liver biopsy before and after the treatment.

Results DR4 was detected in 32 and DR14 in 11 patients; seven possessed both DR4 and DR14. DR4 was more frequent in AIH patients than in the general population (67% vs. 22%), while DR14 was comparably frequent between them (23% vs. 17%). Overall, biochemical response was achieved in 43 (90%) of the 48 patients. The sustained biochemical response to a maintenance prednisolone dose < 10 mg was gained more frequently in the patients with than without DR14 (10/11 [91%] vs. 10/37 [27%], $P < 0.001$). Marked histological improvement with

a decrease in histology activity index (HAI) score by > 2 points was achieved in 31 of the 32 (97%) biochemical responders. Histological aggravation with an increase in HAI score occurred in 4 of the 16 (25%) patients without biochemical response (non-responders and relapsers combined), but in none of the 32 responders.

Conclusion Long-term immunosuppressive treatment can improve the outcome of Japanese patients with AIH, and DR14 is associated with excellent biochemical response.

Keywords Hepatitis · Autoimmune-HLA-DR-corticosteroids-biopsy · Needle

Introduction

Autoimmune hepatitis (AIH) is the inflammation of hepatocytes of unknown etiology and characterized by histological hallmark of interface hepatitis with infiltration of lymphocytes in the portal area [1–3]. Female preponderance, various auto-antibodies and hyper- γ -globulinemia, as well as excellent response to immunosuppressive therapies, are prominent clinical features. AIH is sub-grouped into types 1–3 by the age of onset, severity of disease, and autoantibody profiles [3]. Loss of immunotolerance to self-antigens expressed on hepatocytes is implicated in the pathogenesis of AIH, in the background of major histocompatibility complex (MHC) genes represented by HLA-DR alleles [4].

The disease entity of AIH is not uniform and influenced by geography and ethnicity, in which HLA-DR types play a major role. For the purpose of dealing with a broad clinical spectrum of AIH, diagnostic criteria were proposed by the International Autoimmune Hepatitis Group (IAIHG) in 1993 [5], and they were modified in 1999 [6]. In Japan, an

Y. Suzuki (✉) · K. Ikeda · M. Hirakawa · Y. Kawamura · H. Yatsuji · H. Sezaki · T. Hosaka · N. Akuta · M. Kobayashi · F. Suzuki · S. Saitoh · Y. Arase · H. Kumada
Department of Hepatology, Toranomon Hospital, 1-3-1, Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Japan
e-mail: suzunari@interlink.or.jp

M. Kobayashi
Research Institute for Hepatology, Toranomon Hospital, Tokyo, Japan

Y. Miyakawa
Miyakawa Memorial Research Foundation, Tokyo, Japan

indigenous scoring system for defining AIH was established in 1996 [7]. It has allowed to distinguish AIH from other autoimmune liver disease, such as primary biliary cirrhosis and primary sclerosing cholangitis [8]. Although the treatment response differs in AIH patients with distinct DR profiles, aggressive immunosuppressive treatments with precaution to avoid side-effects can prevent histological deterioration toward favorable long-term outcomes [9, 10].

Since by far the most patients with AIH can merit from immunosuppressive treatment, an effective therapy for an appropriate duration is the primary goal of physicians. AIH can run a rapid course accompanied by cirrhosis in some cases, particularly in young male patients [11], when they fail to receive a therapeutic intervention [12]. Some patients relapse after treatment, often accompanied by rapid deterioration in the liver histology [13]; they need utmost care for timely and effective treatment.

In order to examine a long-term prognosis of AIH, 48 patients with the definite diagnosis of AIH were treated with long-term corticosteroid for up to 28 years, and followed for biochemical and histological responses to treatment, with a special reference to their HLA-DR profiles.

Methods

Patients

During 28 years from 1979 to 2007, 118 patients with AIH type-1 visited the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. Of these patients, 78 (66%) fulfilled the definite diagnostic criteria defined by IAIHG [6], while the remaining 40 (34%) did those of probable AIH. All of the patients were negative for antibodies to liver kidney micosome-1 (anti-LKM-1), and they were classified into AIH type-1. They had a median age of 52 years (range: 19–64 years), and included 45 (67%) women. There were four patients who underwent transient and moderate increases in the serum level of alanine aminotransferase (ALT), and they were followed without treatment. The remaining 60 patients received corticosteroid therapy and were followed for biochemical response during the median of 9 years (range: 5–28 years). Of these patients, 48 (70%) were included in this study, and received serial liver biopsies under laparoscopy for the evaluation of histological improvement. None of them had ongoing infection with hepatitis B or C virus, or possessed antibody to human immunodeficiency virus type-1. The study protocol conformed to the 1975 Declaration of Helsinki, and was approved by the Ethics

Committee of the Toranomon Hospital. Every patient or his/her next of kin gave an informed consent on the purpose of this study.

Serological Tests

Autoantibodies as well as immunoglobulins of IgG and IgM classes were determined by enzyme immunoassay (EIA). Antinuclear antibodies (ANA) were determined by indirect immuno-fluorescence with Hep-G2 cells, and anti-smooth muscle antibodies as well as anti-LKM-1 by indirect fluorescence on cryostat sections of rat organs by the standard procedure. Hepatitis B surface antigen (HBsAg) was determined by radioimmunoassay, antibody to hepatitis C virus (anti-HCV) by EIA of the third generation, and HCV RNA by reversed-transcription polymerase chain reaction (RT-PCR).

HLA Typing

HLA typing was performed by serological methods, and confirmed by PCR-MPH (microplate hybridization) for patients with inconclusive results [14].

Prednisolone Treatment and Biochemical Response

As soon as the diagnosis of AIH was established, patients received 30–60 mg prednisolone daily and were followed for transaminase levels during a mean follow-up period of 5 years (range: 5–28 years). Aminotransferase levels were monitored monthly, and the dose of prednisolone was reduced by 10–15% for the patients in whom ALT levels were normalized to below 40 U/l for 3 months or longer. The response was judged 6 months after the normalization of ALT. Complete response was defined by the normalization of transaminase levels with a maintenance dose of ≤ 10 mg prednisolone daily; partial response by that with >10 mg prednisolone (up to 20 mg); and no response by the failure in normalizing transaminase levels with a maintenance dose of prednisolone (10–20 mg). Relapse was an exacerbation with increase in ALT levels exceeding 80 U/l ($2 \times$ upper limit of normal) after they had been normalized by a maintenance dose.

Laparoscopic and Histological Examinations

Patients received liver biopsy under laparoscopy before and after the treatment with an interval of 5 years with a minor patient-to-patient variation. Biopsied liver specimens were stained for silver for evaluating fibrosis and with D-periodic acid Schiff (PAS) for examining inflammatory changes.

Statistical Analysis

Categorical variables were compared between groups by the χ^2 test and Fisher's exact test, and non-categorical variables by the Mann–Whitney's *U* test.

Results

Baseline Characteristics of AIH Patients

Table 1 lists the baseline characteristics of the 48 patients with AIH for whom HLA typing was performed and who had received a long-term immunosuppressive therapy (median 9 years [range: 5–28 years]) while they were monitored for biochemical and histological responses. Frequencies of HLA-DR are shown in Fig. 1. DR4 predisposing Japanese patients to AIH [15, 16] was detected in 32 of the 48 (67%) patients, DR8 in nine (19%), DR14 in 11 (23%) and DR15 in 16 (33%) of the 48 AIH patients.

Biochemical Responses of AIH Patients with Reference to HLA Types

Biochemical response with the normalization of aspartate aminotransferase (AST) and ALT levels was achieved in 43 of the 48 (90%) patients after the initial aggressive treatment with corticosteroids (30–60 mg/day of prednisolone) followed by a small maintenance dose (10 mg/day or less). However, 16 of the 43 (37%) responders required occasional increased doses (20 mg/day or more) for the treatment of

Table 1 Baseline characteristics of the 48 patients with AIH

Features	Normal range	
Age (years)	Not applicable	52 (22–71)
Men	Not applicable	10 (21%)
AST (IU/l)	11–38	93 (16–1,550)
ALT (IU/l)	6–50	110 (16–2,640)
ALP (IU/l)	117–350	282 (128–949)
γ -GTP (IU/l)	9–109	84 (15–651)
γ -Globulin (g/dl)	0.76–1.76	2.27 (1.36–4.59)
IgG (mg/dl)	870–1,700	2,632 (1,340–2,632)
ANA (x)	<80	640 (0–10,240)
Fibrosis stage	Not applicable	
F ₀		0
F ₁		19 (40%)
F ₂		17 (35%)
F ₃		10 (21%)
F ₄		2 (4%)

Data are expressed by the median with the range in parentheses or the number with percentage in parentheses

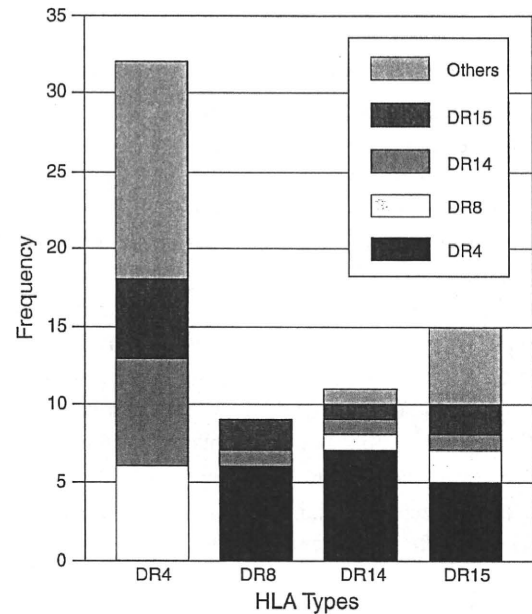


Fig. 1 HLA-DR alleles in the 48 patients with AIH. The allele in the other chromosome is shown in patients with DR4, DR8, DR14, and DR15

Table 2 Biochemical response in AIH patients with or without the DR14 allele

HLA-DR	Number (n = 48)	Biochemical response			Relapse
		Complete	Partial	None	
DR14	11 (23%)	10 (91%)*	1 (9%)	0	0
Non-DR14	37 (77%)	10 (27%)	11 (29%)	5 (14%)	11 (28%)
DR4	32 (67%)	11 (34%)	7 (22%)	3 (9%)	11 (34%)
DR8	9 (19%)	3 (33%)	2 (22%)	1 (11%)	3 (33%)
DR15	15 (31%)	6 (40%)	7 (47%)	1 (7%)	1 (7%)
Others	3 (6%)		1 (33%)	2 (67%)	

Transaminase levels were normalized with a maintenance dose of ≤ 10 mg prednisolone in complete responders and with that of >10 mg in partial responders. Relapse was an exacerbation of transaminase levels after they had been normalized by a maintenance dose

* $P < 0.001$ vs. non-DR14

hepatitis flares. Response differed in patients with distinct HLA-DR types (Table 2). Complete biochemical response was more frequent in the patients with than without DR14 (10/11 [91%] vs. 10/37 [27%], $P < 0.001$).

Relationship Between Biochemical and Histological Responses to Prednisolone Therapy in the 48 Patients with AIH

Histological follow-ups were performed in the 48 patients, and the HAI score markedly improved in 42 (88%), moderately improved in two patients (4%) and worsened in the remaining four (8%) (Table 3). Marked histological

Table 3 Relationship between biochemical and histological responses to prednisolone therapy in the 48 patients with AIH

Biochemical Response	Number (<i>n</i> = 48)	Histological response		
		Marked	Moderate	Worsened
Response	32	31 (97%)	1 (3%)	0
Complete	20	19 (95%)	1 (5%)	0
Partial	12	12 (100%)	0	0
No Response	5	2 (40%)	1 (20%)	2 (40%)
Relapse	11	9 (82%)	0	2 (18%)

Histology activity index (HAI) score decreased by ≥ 2 points in marked response and by 1 point in moderate response

improvement was accomplished in 31 of the 32 (97%) responders, while it was achieved in two of the four (50%) non-responders and nine of the 11 (82%) relapsers. Histology worsened in four of the 16 (25%) patients without biochemical response (non-responders and relapsers combined), but in none of the 32 responders. Changes in the total HAI score as well as respective scores for specific histological parameters (periportal with/without bridging necrosis; intralobular degeneration with focal necrosis; portal inflammation; and fibrosis) are shown in Table 4. The gain in total HAI score was due to an increase in inflammation and not attributed to aggravation of fibrosis in each of them.

Histological Responses of AIH Patients with Reference to HLA

Table 5 compares histological responses between the patients with and without DR4. Although the pretreatment HAI score was somewhat higher in the patients with than without DR14 (9.8 ± 3.5 vs. 7.9 ± 3.3 , $P = 0.092$), it improved to comparable extents in both of them after treatment (4.5 ± 0.9 vs. 4.7 ± 2.5). Thus, the marked histological response with a decrease in HAI score ≥ 2 was no different between the patients with and without DR14

Table 4 Changes in the total HAI score and those in respective parameters in the four patients in whom histology worsened after prednisolone treatment

	Total HAI score (scores for each parameter ^a)	
	Before treatment	After treatment
Patient 1	6 (1, 1, 1, 3)	8 (1, 3, 1, 3)
Patient 2	3 (0, 1, 1, 1)	6 (1, 3, 1, 1)
Patient 3	6 (1, 1, 1, 3)	8 (1, 3, 1, 3)
Patient 4	13 (3, 3, 3, 4)	15 (4, 4, 3, 4)

^a Four histological parameters were graded, including periportal with/without bridging necrosis; intralobular degeneration with focal necrosis; portal inflammation; and fibrosis

Table 5 Histological response in AIH patients with or without DR14

HLA-DR	Number	Histological improvement		
		Marked	Moderate	Worsened
DR14	11 (23%)	10 (91%)	1 (9%)	0
Non-DR14	37 (77%)	32 (86%)	1 (3%)	4 (11%)
DR4	32 (67%)	27 (84%)	1 (3%)	4 (13%)
DR8	9 (19%)	8 (89%)	1 (11%)	0
DR15	15 (31%)	3 (93%)	0	1 (7%)
Others	3 (6%)	2 (67%)	0	1 (33%)

(10/11 [91%] vs. 32/37 [86%], $P = 0.697$). Improvement in the histology was mostly due to changes in the necro-inflammatory grade; there were few changes in the fibrosis grade from the baseline values.

Figure 2 illustrates clinical and histological courses of a representative patient (female, 50 years old, HLA-DR4/DR14) who received eight laparoscopies and seven liver biopsies during the follow-up for 20 years. Before she received corticosteroid therapy, liver histology had already progressed to cirrhosis, and she had to undertake sclerotherapies for the treatment of esophageal varices. She had to receive 10–30 mg prednisolone during initial few years for the treatment of several hepatitis flares. Thereafter, her liver function improved remarkably and had remained within normal limits by a maintenance dose of ≤ 10 mg prednisolone through 17 years until the last follow-up. Remarkably, she gained improvement not only in the inflammation grade but also in the fibrosis stage. Serial laparoscopic and histological findings of her liver are demonstrated in Fig. 3. In other AIH patients, also, aggressive immunosuppressive therapy prevented histological progression and gained improvement in their long-term outcomes, even though their responses to prednisolone differed.

Discussion

In the present study, HLA typing was performed in 48 of the 78 (62%) patients with the definite diagnosis of AIH type-1. They had been followed-up during a long-term corticosteroid treatment, with liver biopsies performed as frequently as possible, and histological and biochemical responses were correlated with HLA types. DR14, which has not gained attention in AIH, was detected in 11 of the 48 (23%) patients. Remarkably, the sustained biochemical response was achieved more frequently in the AIH patients with than without DR14 (10/11 [91%] vs. 10/37 [27%], $P < 0.001$).

The association of HLA types and AIH are under regional influence. DR3 and DR4 are the main HLA

Fig. 2 Clinical course of a patient with AIH (female, 45 years old with HLA-DR4/DR14) who had been followed for 20 years. Doses of prednisolone are indicated at the top, and appearances of the liver surface on laparoscopies, as well as fibrosis stage and inflammation grade on liver biopsies, are shown in the middle. During the initial few years, she received up to 30 mg prednisolone per day for treatment of several hepatitis flares. Thereafter, her liver function improved remarkably and had stayed within normal limits through 17 years with a maintenance prednisolone dose ≤ 10 mg

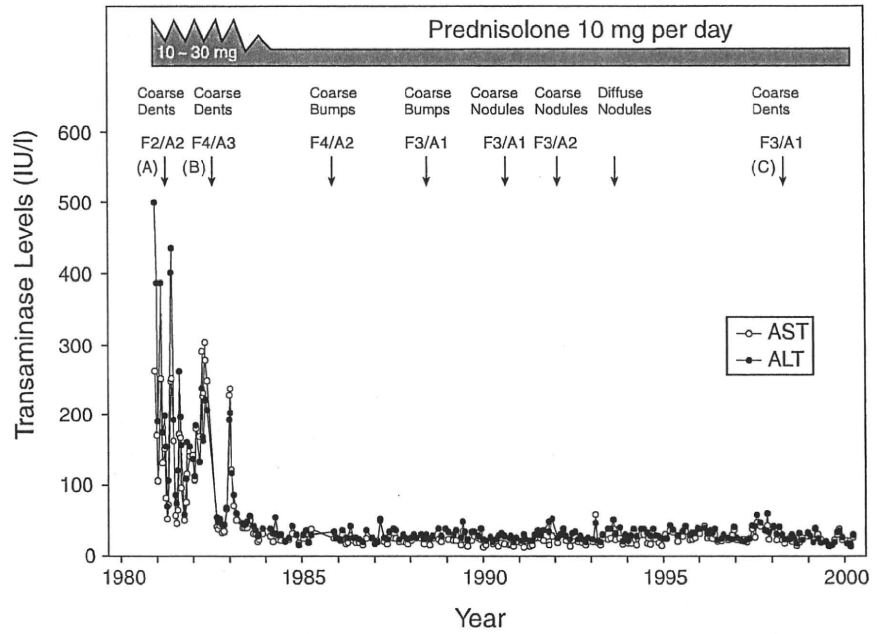
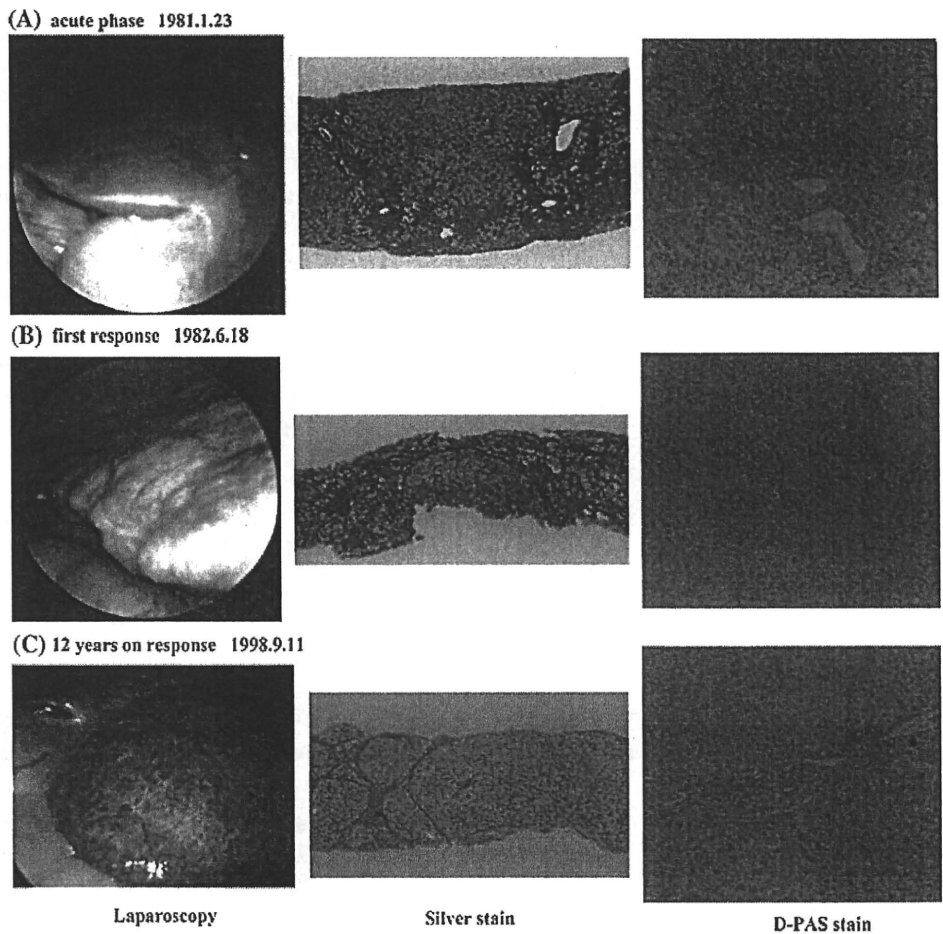


Fig. 3 Laparoscopic findings and histological changes in the patients with AIH. The patient presented in Fig. 2 was examined at three time points (a, b, and c in Fig. 2). Laparoscopic findings were improved since she responded to prednisolone since June, 1982. Histologically, typical submassive necrosis and interface-hepatitis were found in the first biopsy (a). Since she responded, necroinflammatory changes improved, however (b and c). Laparoscopic findings are shown in right row, low-power fields ($\times 20$) by silver staining in the middle row; and high-power fields ($\times 200$) by D-PAS staining



susceptibility alleles among Caucasoid Northern Europeans and North Americans, and 84% of adult patients have either or both of these alleles [17, 18]. In contrast, the principal susceptibility allele for AIH in Japan is DR4 [15, 16]; DR3 is detected in none of them, however. DR4 is also frequent in adult patients in Argentina and Mexico [19, 20], while DRB13 prevails in Argentine and Brazilian children with AIH [21, 22]. DR4 is associated with better response and fewer relapse than DR3 in AIH patients from Western countries [1]. However, there have been no reports on the association of HLA types with treatment response in patients with AIH in Japan. In the present study, DR4 was detected in 32 of the 48 (67%) Japanese patients with AIH, with a frequency comparable to those in previous reports [15, 16]; DR4 was more common in AIH patients than in the general Japanese population (67 vs. 22%) [23]. In contrast, DR14 was comparably frequent in AIH patients and the general population of Japan (23 vs. 17%) [23]. Thus, DR4 would predispose the Japanese population to the development of AIH, while DR14 would not, albeit DR14 would increase the response to corticosteroids in AIH patients.

On the basis of DR4 that is more frequent in the individuals with than without DR3, these alleles have been regarded to behave independently and reciprocally toward the susceptibility for AIH. Such a possibility has been evaluated in peripheral blood mononuclear cells and lymphocytes infiltrating in the liver [24]. Liver lymphocytes are sensitized with hepatocytes or hepatic autoantigens. Even among inflammatory cells infiltrating the portal area, CD4+ lymphocytes predominate in the patients with than without AIH. These lines of evidence implicate the class-II MHC in the pathogenesis of AIH, of which DR4 and DR15 would play major roles in Japan. In the patients with AIH who are positive for LKM-1 antibodies, Th1 cells dominate in the cytokine production assay with a T-cell line specific for LKM-1 [25]. Combined, CD4+ lymphocytes would be crucially required in the manifestation of AIH by interacting with class-II MHC antigens.

In conclusion, the association of MHC class-II antigens with biochemical and histological responses to immunosuppressive treatment was evaluated in Japanese patients with AIH, for predicting their long-term outcomes. On the basis of the results obtained, DR14 would be associated with favorable treatment response in Japanese patients with AIH, which needs to be confirmed in an extended series of patients. The validity of such an assumption will be evaluated by in vitro studies, which are underway.

Acknowledgments This study was supported in part by grants from the Ministry of Health, Labour and Welfare of Japan.

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<短 報>

C 型慢性肝炎に対するペグインターフェロンとリバビリン併用療法における NS3-4A プロテアーゼ阻害剤 (Telaprevir) 併用 12 週間治療の ウイルス学的効果の検討

瀬崎ひとみ*、鈴木 文孝、芥田 憲夫、平川 美晴、川村 祐介
八辻 寛美、保坂 哲也、小林 正宏、鈴木 義之、斎藤 聡
荒瀬 康司、池田 健次、熊田 博光

緒言：現在、C 型慢性肝炎に対する治療はペグインターフェロン (PEG-IFN) とリバビリンの併用療法が標準治療法となっているが、海外においては新規の抗 HCV 薬である NS3-4A protease inhibitor (Telaprevir) の強力な HCV 増殖抑制作用が報告され¹⁾、PEG-IFN とリバビリンとの 3 者併用療法により治療効果が飛躍的に改善することが明らかにされてきている。そこで今回我々は、genotype 1 型、高ウイルス量の C 型慢性肝炎患者に対して PEG-IFN α -2b とリバビリンの併用療法に Telaprevir を併用した 3 者併用 12 週間治療のウイルス学的効果を検討した。

対象と方法：対象は、genotype 1b、高ウイルス量の症例で、当院において 2008 年 5 月から 2008 年 7 月までに PEG-IFN α -2b とリバビリン治療に Telaprevir を併用する 3 者併用 12 週間治療を施行することに同意した初回治療例の 10 例である。男性 4 例、女性 6 例、年齢は 36-64 歳 (中央値 51 歳) であった。Telaprevir は無作為に 2 群に分類され、A 群は 1 回 750 mg、B 群は 1 回 500 mg で 8 時間ごとに 3 回投与された。投与中の HCV RNA の陰性化を TaqMan PCR 法にて評価し、さらに 12 週併用療法終了後 24 週経過観察した時点での完全著効 (SVR) 率を評価した。

結果：治療中および治療終了後の経過を Fig. 1 に示す。12 週間の治療を完遂できたのは 5 例 (50%) であった。4 例はヘモグロビン値の低下、1 例は倦怠感により治療

中止となった。しかしながら、HCV RNA は全例で治療中に陰性化を認め、陰性化時期は 2~5 週 (中央値 2 週) と非常に早期であった。Case 1~5 は 12 週までに中止となったが、このうち 2 週目で陰性化した 3 例は 5 週目、7 週目、10 週目に治療を中止したにもかかわらず SVR となった。Case 6~10 は 12 週間投与を完遂した症例であるが、5 週目で陰性化した 1 例を除き、4 例が SVR に至った。最終的な SVR 率は全体で 7/10 例 (70%) と高率であった。

Telaprevir の用量は A 群 6 例、B 群 4 例に割り付けられた。中止率は両群とも 50% であり、SVR 率は A 群 4/6 例 (66.7%)、B 群 3/4 例 (75%) と両群間で治療効果、副作用に差は認めなかった。

男女別にみると、男性 3/4 例 (75.0%)、女性 4/6 例 (66.7%) であり、50 歳以上の女性のみでも、3/3 例 (100%) と高率に SVR を得られた。

HCV core 領域 70 番目のアミノ酸変異の有無から治療効果をみると、wild type の症例は 5/6 例 (83.3%)、mutant type では 2/4 例 (50%) が SVR に至った。

考察：NS3-4A protease inhibitor (Telaprevir) を用いた PEG-IFN とリバビリンとの 3 者併用療法は非常に抗ウイルス効果が高く、以前我々は、genotype 1b 型の慢性肝炎症例に対する 3 者併用 12 週間投与における治療中の HCV RNA 動態を検討し、2 週目で 50%、4 週目で 79%、8 週目で 94%、12 週目で 100% に HCV RNA の陰性化を認めたことを報告した²⁾。今回は、この症例のうち初回治療例について 24 週間の経過観察終了後の最終的な治療成績を検討した。その結果、初回治療例に対しては 12 週間の治療でも SVR に至る症例が 70% に達し、ウイルス排除を目的とした治療として有用であることが判明した。これは欧米の PROVE1³⁾ および

虎の門病院肝臓センター

*Corresponding author: hitomis@mx1.harmonix.ne.jp

\$ 利益相反申告：瀬崎ひとみ、株式会社田辺三菱製薬

<受付日 2010 年 2 月 17 日><採択日 2010 年 5 月 18 日>

索引用語 : C 型慢性肝炎, リバビリン併用療法,
NS3-4A プロテアーゼ阻害剤

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Med Virol 2010; 82: 575—582

英文要旨

The efficacy of virological response in treatment-naïve patients with chronic hepatitis C treated by NS3-4A protease inhibitor (telaprevir), pegylated interferon and ribavirin for 12 weeks

Hitomi Sezaki*, Fumitaka Suzuki,
Norio Akuta, Miharuru Hirakawa,
Yusuke Kawamura, Hiromi Yatsuji,
Tetsuya Hosaka, Masahiro Kobayashi,
Yoshiyuki Suzuki, Satoshi Saitoh,
Yasuji Arase, Kenji Ikeda,
Hiromitsu Kumada

We investigated the efficacy of the triple treatment with telaprevir, pegylated interferon (PEG-IFN) and ribavirin for 12 weeks in treatment-naïve patients infected with hepatitis C virus (HCV) genotype 1b and high baseline viral loads. All of 10 cases became HCV-RNA negative during treatment. SVR rate attained to a high rate, 70% (7/10). Especially, SVR rate of females over 50 years old attained 100% (3/3). HCV RNA was lost from serum rapidly in patients infected with HCV-1b in high viral loads, and SVR rate of the triple treatment for 12 weeks was high. Our results suggested that triple treatment with telaprevir, PEG-IFN and ribavirin could improve the efficacy in treatment-naïve patients.

Key words: chronic hepatitis C,
interferon plus ribavirin
combination therapy,
NS3-4A protease inhibitor

Kanzo 2010; 51: 394—396

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

*Corresponding author: hitomis@mx1.harmonix.ne.jp

<短 報>

核酸アナログ未使用のB型慢性肝炎症例へのエンテカビル治療中に rtA181T変異ウイルスが増殖した1症例

八辻 寛美^{1)*} 鈴木 文孝¹⁾ 平川 美晴¹⁾ 川村 祐介¹⁾ 瀬崎ひとみ¹⁾
 保坂 哲也¹⁾ 芥田 憲夫¹⁾ 小林 正宏¹⁾ 鈴木 義之¹⁾ 斉藤 聡¹⁾
 荒瀬 康司¹⁾ 池田 健次¹⁾ 岩崎 里美²⁾ 峰田 理恵²⁾ 綿引 祥子²⁾
 小林万利子²⁾ 熊田 博光¹⁾

緒言：核酸アナログ未使用のB型慢性肝炎患者へのエンテカビル治療中に、既報のエンテカビル耐性ウイルスが出現していないにもかかわらず、viral reboundを生じた症例を経験したため、報告する。

症例：51歳女性。1978年にB型慢性肝炎と診断され、2008年6月よりエンテカビル(0.5 mg/日)治療を開始した。治療開始時HBV-DNA 7.2 log copies/ml, HBeAg陽性, genotype Cであった。2009年2月HBV-DNA 2.5 log copies/mlまで下がるも、その後2009年4月HBV-DNA 6.0 log copies/ml, 8月8.2 log copies/mlとviral reboundが出現し、トランスアミナーゼの上昇も認めた(Fig. 1)。

治療開始時および治療中のHBV-DNA polymerase RT領域のアミノ酸配列の比較検討：患者血清から抽出されたHBV-DNAはPCR法にて増幅したのち、direct sequence法にて塩基配列を決定した。クローニング解析もあわせて行った。ダイレクトシーケンスでは核酸アナログ未使用であるにもかかわらず、エンテカビル開始時にrtA181T変異のわずかな混在を認め、クローニング解析では8.5% (3/35クローン)にrtA181T変異を確認した。また治療開始後15カ月ではダイレクトシーケンスにてrtA181T変異の混在の割合が増加しており、クローニング解析にてrtA181T変異は39.5% (17/43クローン)に増加していた。尚、エンテカビル開始時および治療中にrtA181以外の既報のエンテカビ

ル耐性に関するアミノ酸 (rtL180, T184, S202, M204, M250) に変異は認められなかった (Fig. 1)。

考察：今回我々は、エンテカビル投与にてrtA181T変異が増殖した症例を経験した。本症例はエンテカビル投与中にviral reboundを生じ、その際既報のエンテカビル耐性ウイルスは出現せず、治療開始時よりわずかに認められていたrtA181T変異ウイルスが増殖していた。クローニング解析にてrtA181T変異ウイルスは治療開始時8.5%から治療開始15カ月後に39.5%に増加し、他に有意なアミノ酸変異を認めないことから、rtA181T変異がエンテカビル耐性に関与している可能性が考えられた。しかし本症例で出現したrtA181T変異ウイルスのエンテカビル耐性への関与を証明するためには、今後本症例の血清を使用したin vitroの実験にて評価する必要があると考える。また本症例ではviral reboundと同時にトランスアミナーゼ上昇も認められたが、軽度上昇にとどまっているため、現在もエンテカビル治療を継続し厳重にフォローしている。

本症例は、核酸アナログ未使用のB型慢性肝炎症例であったにもかかわらず、エンテカビル治療開始前よりrtA181T変異が存在していた。核酸アナログ未使用症例にラミブジン耐性に関するrtL180M, rtM204V変異が存在するという報告はあるが、本症例のようにrtA181T変異が核酸アナログ使用前に存在したという報告は過去になく、初めての報告である。

rtA181T変異は以前よりアデホビル耐性に関するアミノ酸変異として知られていたが、最近ではラミブジンとアデホビルの交差耐性のある変異であることがわかっている¹⁾。このためrtA181T変異に対してエンテカビルの効果が期待されている。しかし海外からは、ラミブジン耐性ウイルスに対するアデホビル単独治療

1) 虎の門病院肝臓センター

2) 虎の門病院肝臓研究室

*Corresponding author: h-ooga@mx1.harmonix.ne.jp

<受付日2009年12月25日><採択日2010年2月25日>

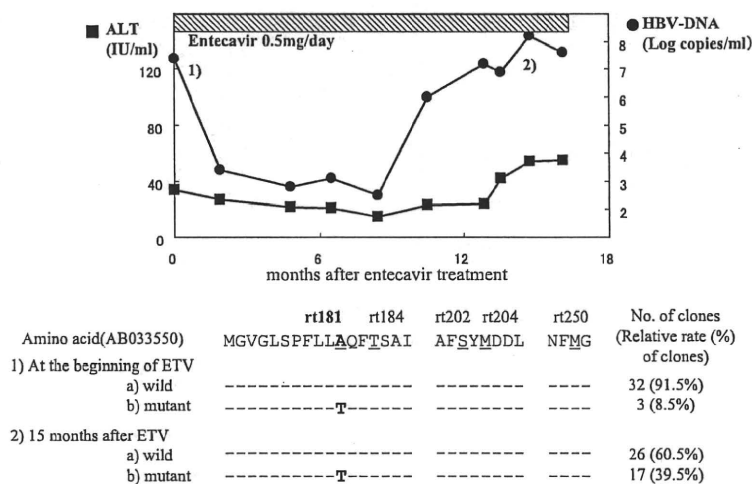


Fig. 1 Clinical course and clonal analysis of samples from patient with viral rebound during entecavir therapy

中に耐性ウイルス (rtA181T/V または N236T 変異ウイルス) が出現した症例は、ラミブジン耐性ウイルスのみの症例に比べ、エンテカビル治療におけるウイルス抑制効果が低いという報告があり²⁾、また本症例のようにエンテカビル治療にて rtA181T 変異ウイルスが増加する症例も存在することから、今後 rtA181T 変異ウイルスに対する治療として、エンテカビル以外の核酸アナログ (テノフォビル, その他新規薬剤等) の有効性も検討していく必要があると考えられる。

索引用語：エンテカビル, 耐性ウイルス, rtA181T

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英文要旨

Increase of rtA181T mutant strains during entecavir therapy for a patient with chronic hepatitis B virus infection

Hiromi Yatsuji^{1)*}, Fumitaka Suzuki¹⁾,
Miharu Hirakawa¹⁾, Yusuke Kawamura¹⁾,
Hitomi Sezaki¹⁾, Tetsuya Hosaka¹⁾,
Norio Akuta¹⁾, Masahiro Kobayashi¹⁾,
Yoshiyuki Suzuki¹⁾, Satoshi Saitoh¹⁾,
Yasuji Arase¹⁾, Kenji Ikeda¹⁾,
Satomi Iwasaki²⁾, Rie Mineta²⁾,
Sachiyo Watahiki²⁾, Mariko Kobayashi²⁾,
Hiromitsu Kumada¹⁾

A 51-year-old Japanese woman with chronic hepatitis B who had never treated with nucleotide analogues was admitted to our hospital and treated with entecavir. In this patient, entecavir successfully reduced the HBV level, but viral and biochemical breakthrough was observed at 10 months after the beginning of therapy. The HBV viral load reached up to 8.2 log copies/ml, but direct sequence analysis showed no LAM and ETV resistant-related mutation (rtT184, S202, M204, M250). Comparison by clonal analysis of samples obtained before and after the viral breakthrough showed the increase of the rtA181T mutant strains (8.5% versus 39.5%). It was considered that the rtA181T mutant

strain in this case might be related to entecavir resistance.

Key words: entecavir, drug-resistant mutant, rtA181T

- 1) Department of Hepatology, Toranomon Hospital
- 2) Department of Research Institute for Hepatology, Toranomon Branch Hospital, Kawasaki

*Corresponding author: h-ooga@mx1.harmonix.ne.jp

Kanzo 2010; 51: 196—198

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<速報>

IL28B と HCV Core aa70 置換との関連

小林万利子^{1)*} 鈴木 文孝²⁾ 芥田 憲夫²⁾ 鈴木 義之²⁾ 瀬崎ひとみ²⁾
 八辻 寛美²⁾ 保坂 哲也²⁾ 小林 正宏²⁾ 川村 裕介²⁾ 平川 美晴²⁾
 荒瀬 康司²⁾ 池田 健次²⁾ 峰田 理恵¹⁾ 岩崎 里美¹⁾ 綿引 祥子¹⁾
 中村 祐輔³⁾ 茶山 一彰⁴⁾ 熊田 博光²⁾

はじめに：C型慢性肝炎の治療法であるPEG-IFN/Rivabirin 併用療法のHCV genotype 1bで高ウイルス量症例では、その排除率が50%台である。この難治症例の治療効果予測因子としてHepatitis C virus NS5A領域のInterferon sensitivity-determining regionやCore領域の70番目, 91番目のアミノ酸置換が有用であることは周知のごとくであったが、近年アメリカ・日本から宿主側因子として*IL28B*のSNPsがPEG-IFN/Rivabirin 併用療法の治療効果予測として有用であると報告¹⁾⁻³⁾されている。今回我々は、C型慢性肝疾患患者のHCV Core aa70と*IL28B*を測定し性差との関連性を検討した。

対象と方法：1997年から2005年までに虎の門病院倫理委員会及びヒトゲノム委員会で承認された同意書を得た患者291人のchromosome 19上の*IL28B*近傍の2つのSNPs (rs8099917 (T/G), rs12979860 (C/T))とHCV Core領域aa70を測定したHCV genotype 1bとした。内訳は、男性177人(年齢：21-82(中央値56歳)、女性114人(年齢：37-82(中央値61歳)であった。

*IL28B*のSNPs (rs8099917, rs12979860)のタイピングはInvador assay, Taqman assayまたはdirect sequencing法にて決定した。rs8099917は290例、rs12979860は289例のタイピング可能であった。HCV Core領域aa70の測定は、PCR-direct sequence法にて測定した。性別とSNPの遺伝子型を検討した。

- 1) 虎の門病院肝臓研究室
- 2) 虎の門病院肝臓センター
- 3) 理化学研究所ゲノム医科学研究センター
- 4) 広島大学大学院医歯薬学総合研究科分子病態制御内科学

*Corresponding author: vj7m-kbys@asahi-net.or.jp
 <受付日2010年3月10日><採択日2010年5月1日>

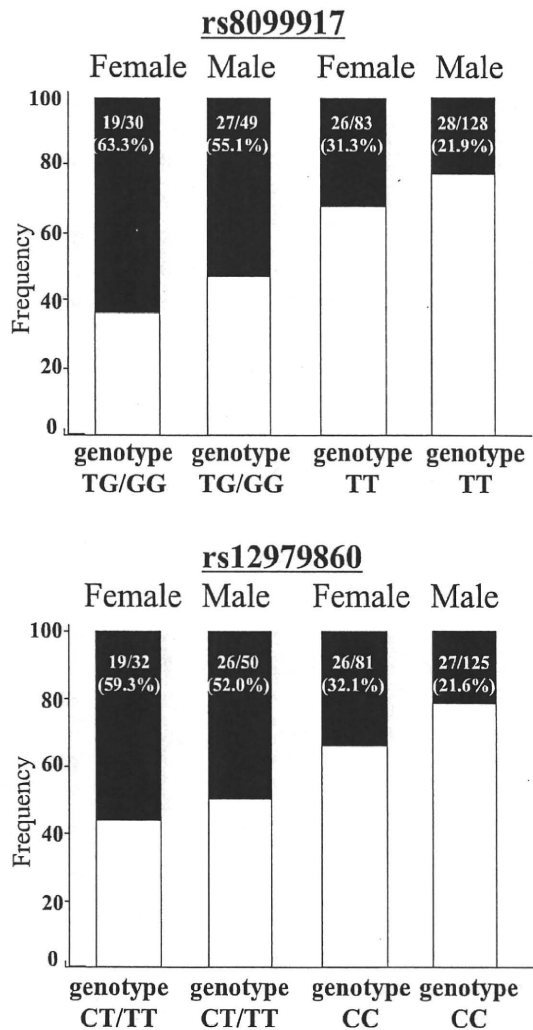


Fig. 1 Relationship between *IL28B* SNPs and amino acid substitution in hepatitis C virus core region in patients with chronic hepatitis C. Black bars represent aa70 mutant (Gln) while white bars represent aa70 wild (Arg)

結果 : Core aa70 置換からみた IL28B の SNP と性差の頻度

rs8099917 に関しては, Core aa70 の Mutant (Gln) がもっとも高頻度に見られたのは genotype TG/GG の女性で 19/30 例 (63.3%), 次いで男性の genotype TG/GG で 27/49 例 (55.1%), 女性の genotype TT で 26/83 例 (31.3%) であり, 最も低率であったのが男性の genotype TT で 28/128 例 (21.9%) であった (Fig. 1).

rs12979860 においても同様の傾向を認め, 女性の genotype CT/TT で 19/32 例 (59.3%), 男性の genotype CT/TT で 26/50 例 (52.0%) であり, 女性の genotype CC で 26/81 例 (32.1%), 男性の genotype CC で 27/125 例 (21.6%) であった (Fig. 1).

考案 : 近年, IL28B 領域の SNPs が C 型肝炎ウイルスの自然排除¹⁾および慢性肝炎の PEG-IFN/Ribavirin 併用療法の治療効果と関連があることが報告された^{2)~3)}. 我々は, ウイルス側の予測因子である Core aa70 置換について性差を加味して SNP の遺伝子型別にその頻度を解析したところ 2 つの SNP で女性のマイナーアレルホモ接合体及びヘテロ接合体群において Core aa70 (Gln) Mutant の頻度がいずれも 50% 台であった. このことは, 高齢の女性は PEG-IFN/Ribavirin 併用療法の治療効果が低い傾向を示すことならぬとの関連が推測され, 女性において Core aa70 は, 経過観察中にメジャークローンとマイナークローンが入れ代わる可能性が示唆された. 今後, 治療効果予測として宿主側因子の一つである IL28B の SNPs と Core aa70 置換の組み合わせにより, より有効な治療効果予測が可能になると思われた.

索引用語 : C 型慢性肝疾患, IL28B, コア領域

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英文要旨

Relationship between SNPs in the IL28B region and amino acid substitutions in HCV core region in Japanese patients with chronic hepatitis C

Mariko Kobayashi¹⁾*, Fumitaka Suzuki²⁾,
Norio Akuta²⁾, Yoshiyuki Suzuki²⁾,
Hitomi Sezaki²⁾, Hiromi Yatsuji²⁾,
Tetsuya Hosaka²⁾, Masahiro Kobayashi²⁾,
Yusuke Kawamura²⁾, Miharuru Hirakawa²⁾,
Yasuji Arase²⁾, Kenji Ikeda²⁾,
Rie Mineta¹⁾, Satomi Iwasaki¹⁾,
Sachiyo Watahiki¹⁾, Yusuke Nakamura³⁾,
Kazuaki Chayama⁴⁾, Hiromitsu Kumada²⁾

IL28 locus polymorphisms have been reported to affect PEG-IFN plus ribavirin combination therapy for patients with genotype 1b hepatitis C virus (HCV) infection. We examined a relationship between IL28B SNPs (rs8099917 and rs12979860) and amino acid substitutions in core region of HCV in patients with genotype 1b chronic hepatitis C. In each SNP, frequency of core aa 70 mutation was higher rate in female patients carrying minor allele than in male or female patients carrying no minor allele. Measurement of IL28B and Core aa70 before treatment is useful in PEG-IFN plus ribavirin therapy.

Key words: IL28B, HCV, core region

Kanzo 2010; 51: 322—323

- 1) Department of Research Institute for Hepatology, Toranomon Hospital, Kawasaki, Japan
- 2) Department of Hepatology, Toranomon Hospital, Tokyo, Japan
- 3) Laboratory for Molecular Medicine, Human Genome Center, The Institute of Medical Science, University of Tokyo, Tokyo, Japan
- 4) Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan

*Corresponding author: vj7m-kbys@asahi-net.or.jp

新しい超高感度 HBs 抗原定量試薬の基礎的・臨床的有用性

新海 登*1 田中 靖人*2 松浦 健太郎*3
可児 里美*4 長沼 初江*5 溝上 雅史*6

Evaluation and Application of a Newly Developed Highly Sensitive HBsAg Chemiluminescent Enzyme Immunoassay for Chronic Hepatitis B Patients

*Noboru SHINKAI*1, Yasuhito TANAKA*2, Kentaro MATSUURA*3,
Satomi KANI*4, Hatsue NAGANUMA*5 and Masashi MIZOKAMI*6*

Aim: A high sensitive chemiluminescent enzyme immunoassay (CLEIA) was developed for quantitative hepatitis B surface antigen (HBsAg) detection by a combination of monoclonal antibodies, each one for a specific epitope of HBsAg, and by improving the conjugation technique (Matsubara, et al. Transfusion 2009). We modified and automated Matsubara's techniques. Our aim is to evaluate the fundamental performance of our assay (prototype) and to investigate the clinical significance of prototype in patients with hepatitis B virus (HBV) infection.

Methods: We used 226 HBsAg-negative samples and 59 HBsAg-positive samples for evaluation of prototype's accuracy, reproducibility, specificity and sensitivity. We examine correlation between the prototype assay and commercial quantitative HBsAg detection assay (the Abbott ARCHITECT). Performance of prototype was compared with the Abbott ARCHITECT in one chronic hepatitis B patient and one patient with HBsAg seroconversion sequentially.

Results: The prototype assay had good accuracy, reproducibility, specificity and sensitivity. There is positive correlation between the prototype and the Abbott ARCHITECT. The sensitivity of the prototype (5mIU/mL) was approximately 10 fold higher than the Abbott ARCHITECT (50mIU/ml). The prototype could detect HBsAg at the HBsAg-negative point by Abbott ARCHITECT in these patients.

Conclusions: Automatic highly sensitive HBsAg CLEIA prototype is convenient and precise assay for HBV monitoring.

[Rinsho Byori 58 : 1078~1084, 2010]

Corresponding author: *Yasuhito TANAKA*, Departments of Virology & Liver unit, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan. E-mail: ytanaka@med.nagoya-cu.ac.jp

【Key Words】 hepatitis B virus (B型肝炎ウイルス), highly sensitive HBsAg chemiluminescent enzyme immunoassay (高感度 HBs 抗原測定), CLEIA (化学発光酵素免疫測定), Lumipulse G1200 (ルミパルス G1200)

受付 2010 年 7 月 20 日・受理 2010 年 9 月 30 日

*1-3 名古屋市立大学大学院医学研究科病態医科学, *2,3 同 医学研究科消化器・代謝内科学,

*2,4,5 同 大学病院中央臨床検査部 (〒467-8601 名古屋市瑞穂区瑞穂町字川澄 1)

*6 国立国際医療研究センター肝炎・免疫研究センター (〒272-0827 市川市国府台 1-7-1)

世界の4億2,000万人以上の人にB型肝炎ウイルス(以下、HBV)は持続感染している¹⁾。現在使用されているB型慢性肝炎患者のHBVのウイルスのモニタリングにはHBV DNA(リアルタイムPCR)、HBe抗原、HBs抗原、HBコア関連抗原がある。

現在、PCR測定をベースにしたHBV DNAが臨床の場合においては特に使用頻度が高いが、最近導入された高感度リアルタイムPCRを用いてもHBV DNA検出には限界があり、特にHBV治療薬として核酸アナログを投与されている患者においては速やかに感度以下となるため、ウイルス複製のモニタリングには必ずしも十分とはいえない。

HBVの持続感染の間、HBs抗原は血中に分泌されるエンベロープ蛋白である。HBs抗原検出は持続感染の指標とされ、ウイルスの転写のテンプレートとなる肝内のcccDNAと関連がある²⁾。また、ペグインターフェロン α の治療を受けているB型慢性肝炎患者において、HBs抗原定量がウイルス複製の代用マーカーとなりうる可能性が出てきている^{3)~5)}。

現在、本邦ではHBs抗原定量測定系としては、アーキテクトHBsAg-QT(Abbott Japan)(測定範囲50~250,000mIU/mL)、HISCL HBsAg(Sysmex)(測定範囲30~2,500,000mIU/mL)が存在し、両社の測定系は相関が良好であり、ともに高感度で広い測定域を有する。最近、松原ら⁶⁾により新しい高感度のHBs抗原定量系が報告された。これはHBV感染初期のHBV検出においてPCR法と同等の感度であるとされている。今回、松原らの検査系を元に新たに開発された超高感度HBs抗原定量測定試薬(富士レビオ株式会社)(以下、「本試薬」とする)の性能(自動化)、特異度、従来のHBs抗原定量測定法との相関、臨床経過のモニタリングにおける有用性について評価した。

I. 方法と試料(または材料)

A. 対象

対象となるサンプルはHBs抗原陰性の3検体、HBs抗原量の異なるHBs抗原陽性の5検体を正確性、同時再現性、日差再現性に用いた。また、226サンプルのHBs抗原陰性検体(うち118サンプルが抗HCV抗体陽性検体)を特異性試験に用いた。アーキテクトHBsAg-QT(Abbott Japan)と本試薬との相関を確認するためにB型慢性肝炎患者からの59検体を用いた。また、経時的にHBs抗原を追跡したB型慢性肝炎1患者から4ポイント、経過中にHBs

抗原が消失したB型慢性肝炎1患者から7ポイントのサンプルを使用した。なお、検体は名古屋市立大学大学院医学研究科倫理委員会の承認を得た上で患者の同意のもと採取した。

B. 本試薬によるHBs抗原定量の測定原理

松原らの報告⁶⁾に準ずるが、変性剤を主成分とする検体処理液で検体を前処理することにより、検体中のHBVエンベロープを破壊する。HBs抗体が存在する場合にはHBs抗原/HBs抗体複合体を乖離させることによりHBs抗原を遊離させる。さらに遊離したHBs抗原の立体構造は変性によりリニアエピトープ化する。これらを特異的に捕捉するモノクローナル抗体を用いて高感度に定量する2ステップサンドイッチ法である。抗体に関しては固相に2種類(外側構造認識:1種類、内側リニア認識1種類)、標識に2種類のモノクローナル抗体を使用しており、それぞれが理論上1対1に対応するように設計されている(Fig. 1)。一次抗体に、従来の外側構造認識“a” determinantのみではなく、内側リニア認識抗体も使用している。

C. 本試薬によるHBs抗原定量の測定方法

測定は全自動化学発光酵素免疫測定装置であるルミバルスG1200(富士レビオ株式会社)を用いた。試薬は1テストごとのカートリッジタイプで、検体処理液と磁性フェライト粒子に結合した前述の抗HBsモノクローナル抗体液(固相抗体液)およびアルカリホスファターゼ標識抗HBsモノクローナル抗体液(標識抗体液)で構成されている。抗体の性状に関しては上記のとおりである。この固相抗体と標識抗体によりHBs抗原をサンドイッチした免疫複合物を形成させ、標識された酵素と化学発光基質(AMPPD)の反応による発光強度を測定する。すなわち、第一反応で検体100 μ Lと検体処理液20 μ Lを固相抗体に加えて10分間反応させ、磁石を用いたB/F分離後、第二反応で標識抗体250 μ Lを固相抗体に加えて10分間反応させる。再度、磁石を用いたB/F分離後、AMPPDを加えて5分間酵素反応が行われる。その後、AMPPDの分解に伴う発光量をルミノメーターで測定し、予め作成された検量線より検体中のHBs抗原濃度が出力される。これらの操作は全て装置内で自動的に行われる。測定範囲は5~150,000mIU/mLであり、判定は5mIU/mL以上を陽性とする。なお、レンジオーバーした検体については200倍希釈による希釈測定が可能である。

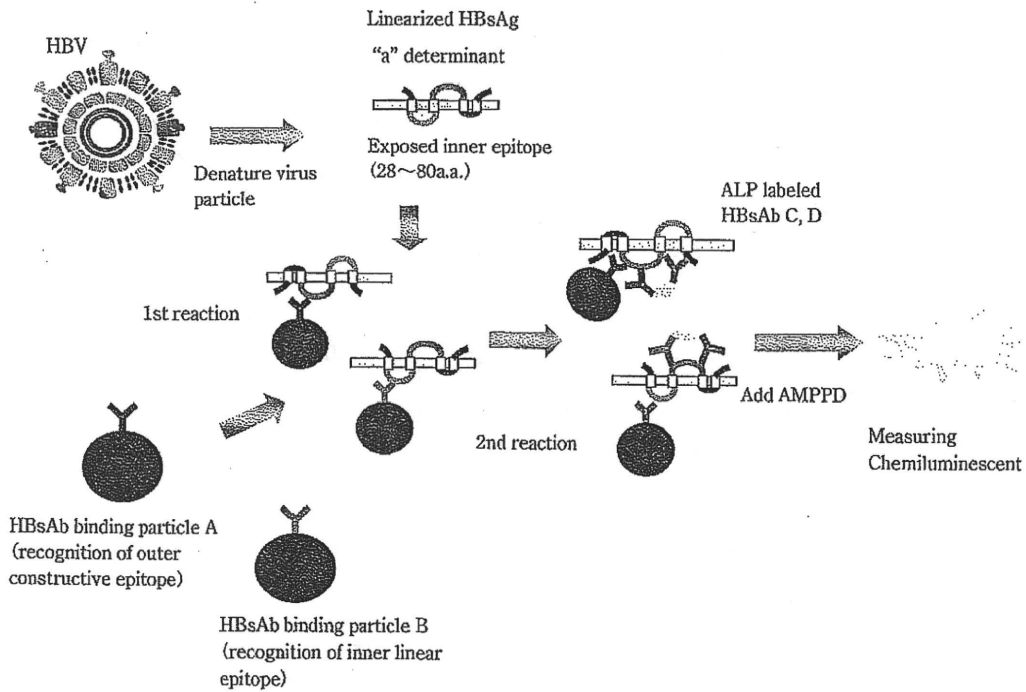


Figure 1 A principle of a newly developed highly sensitive HBsAg chemiluminescent enzyme immunoassay (prototype).

Table 1 Accuracy of highly sensitive HBsAg chemiluminescent enzyme immunoassay (prototype)

Samples	actual value measured by prototype (mIU/ml)	per control value (%)
N1	1.8	—
N2	0.4	—
N3	0.1	—
LL	47.7	96.7
L	538.5	101.8
M	6,413.3	102.5
H	56,872.0	101.6
HH	342,672.5	99.9

II. 結 果

本試薬を①正確性, 同時再現性, 日差再現性, ②特異性試験, ③従来のHBs抗原定量測定法としてアーキテクトHBsAg-QT(アボットジャパン株式会社)(the Abbott ARCHITECT HBsAg-QT.)との相関について評価した。

A. 正確性

①正確性を検討するために陰性検体3例(N1~N3)とHBs抗原量の異なるHBV陽性検体5例(LL,

L, M, H, HH)の8検体を測定した。HBV陽性検体の管理値(control value)は本試薬3ロットを用いて, 各6重測定を行い, その平均値として設定された。測定値(actual value)は, 管理値と比較してほぼ100%の結果が得られた(Table 1)。②同時再現性についても, 前述のHBV陽性検体5例(LL, L, M, H, HH)を用いてそれぞれ6重測定したところ変動係数は5%以下と良好であった(Table 2)。③日差再現性に関しても, 前述のHBV陽性検体5例(LL, L, M, H, HH)を用いて6日測定を施行した。変動係数は