研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	頁	出版年
Hattori J, Okumura N, Yamazaki Y, Uchiyama M, Hamaguchi M, Nishiyama Y, Kaneda T	Beneficial effect of GB virus C co-infection in human immunodeficiency virus type-1-infected individuals.	Microbiol. Immunol.	51	193-200	2007
高橋昌明、藤崎誠一郎、伊部史朗、久高祐一、奥村直哉、平野淳、鈴木達男、横幕能行、間宮均人、濱口元洋、金田次弘	HIV・HCV 重複感染に対するリバビリン併用ペグ・インターフェロン療法により CD4,CD8 陽性リンパ球数が減少した 1 症例.	新薬と臨床	56	112-115	2007
Fujisaki S, Fujisaki S, Ibe S, Asagi T, Itoh T, Yoshida S, Koike T, Oie M, Kondo M, Sadamasu K, Nagashima M, Gatanaga H, Matsuda M, Ueda M, Masakane A, Hata M, Mizogami Y, Mori H, Minami R, Okada K, Watanabe K, Shirasaka T, Oka S, Sugiura W, Kaneda T	Performance and quality assurance of genotypic drug-resistance testing for human immunodeficiency virus type 1 in Japan.	Jpn. J. Infect. Dis.	60	113-117	2007

藤崎誠一郎、藤崎彩恵子、伊部史朗、浅黄司、伊藤俊広、吉田繁、小池隆夫、大家正泰、渡邊香奈子、正兼亜季、上田幹夫、潟永博之、松田昌和、貞升健志、長島真美、岡田清美、近藤真規子、秦 眞美、溝上泰司、森 治代、南 留美、白阪琢磨、岡慎一、杉浦 亙、金田次弘	日本における HIV-1 遺伝子型薬剤耐性検査のコントロールサーベイ	日本エイズ学会誌	9	136-146	2007
Takahashi M, Ibe S, Kudaka Y, Okumura N, Hirano A, Suzuki T, Mamiya N, Hamaguchi M, Kaneda T.	No observable correlation between central nervous system side effects and EFV plasma concentration in Japanese HIV-1 infected patients treated with EFV containing HAART.	AIDS Res. Hum. Retroviruses	23	983-987	2007
平野 淳、奥村直哉、久高祐一、寺畑奈美、高橋昌明、坂野和英、横幕能行、安岡彰、間宮均人、濱口元洋、金田次弘	サイトメガロウイルス感染症を発症した日本人エイズ患者に対するバルガンシクロビルの効果および安全性についての評価	日本病院薬剤師会雑誌	43	1397-1399	2007
Takahashi M, Kudaka Y, Okumura N, Hirano A, Banno K, Kaneda T.	Determination of plasma tenofovir concentrations using a conventional LC-MS method.	Biol. Pharm. Bull.	30	1784-1786	2007

Takahashi M, Kudaka Y, Okumura N, Hirano A, Banno K, Kaneda T.	The validation of plasma darunavir concentrations determined by the HPLC method for protease inhibitors.	Biol. Pharm. Bull.	30	1947-1949	2007
小柏 均、永井裕美、近藤恭子、加堂真由、伊部史朗、一三武二郎、玉村和規、間宮均人、金田次弘	リアルタイム PCR 法による *Pneumocystis jirovecii* 迅速定量法の確立	医学検査	56	1527-1534	2007
Takahashi M, Kudaka Y, Okumura N, Hirano A, Banno K, Kaneda T.	Pharmacokinetic parameters of lopinavir determined by moment analysis in Japanese HIV type 1-infected patients.	AIDS Res. Hum. Retroviruses	24	114-115	2008
Ibe S, Hattori J, Fujisaki S, Shigemi U, Fujisaki S, Shimizu K, Nakamura K, Kazumi T, Yokomaku Y, Mamiya N, Hamaguchi M, Kaneda T.	Trend of drug-resistant HIV type 1 emergence among therapy-naive patients in Nagoya, Japan: an 8-year surveillance from 1999 to 2006.	AIDS Res. Hum. Retroviruses	24	7-14	2008
濱口元洋	免疫再構築症候群とその対応	日本エイズ学会誌	9	91-101	2007
Gatanaga H, Hayashida T, Tsuchiya K, Yoshino M, Kuwahara T, Tsukada H, Fujimoto K, Sato I, Ueda M,	Successful efavirenz dose reduction in HIV type 1-infected individuals with cytochrome P450 2B6 *6 and *26	Clin. Infect. Dis.	45	1230-1237	2007

Horiba M, Hamaguchi M, Yamamoto M, Takata N, Kimura A, Koike T, Gejyo F, Matsushita S, Shirasaka T, Kimura S, Oka S					
Gatanaga H, Ibe S, Matsuda M, Yoshida S, Asagi T, Kondo M, Sadamasu K, Tsukada H, Masakane A, Mori H, Takata N, Minami R, Tateyama M, Koike T, Itoh T, Imai M, Nagashima M, Gejyo F, Ueda M, Hamaguchi M, Kojima Y, Shirasaka T, Kimura A, Yamamoto M, Fujita J, Oka S, Sugiura W.	Drug-resistant HIV-1 prevalence in patients newly diagnosed with HIV/AIDS in Japan.	Antiviral Res.	75	75-82	2007

Ⅳ. 研究成果の刊行物・別刷

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Beneficial Effect of GB Virus C Co-Infection in Human Immunodeficiency Virus Type 1-Infected Individuals

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Abstract: **Several reports have documented a better prognosis for HIV-1-infected patients co-infected with GBV-C, while other reports have contradicted such findings with the result that this issue remains controversial. We attempted to clarify the complicated status of the effect of GBV-C co-infection on HIV-1-infected patients. GBV-C RNA was detected in 37 samples in 182 HIV-1-infected patients (20.3%) using RT/nested PCR. Of these, 3 were determined to be GBV-C genotype 1, 12 were genotype 2, and the remaining 22 were genotype 3. The GBV-C viral load quantified by real-time PCR ranged from 7.8×10^3 to 3.3×10^6 copies/ml. Weakly negative correlation was observed between GBV-C viral load and HIV-1 viral load in 19 HAART-naïve patients, indicating that a higher GBV-C viral load is associated with a greater suppression of HIV-1 replication. A previously published *in vitro* study suggested that GBV-C infection would induce up-regulation of RANTES, leading to suppression of HIV-1 replication. However, in our present study, the blood RANTES level was significantly lower in the GBV-C co-infected group than in the uninfected group (190–9,959 vs. 264–31,038 pg/ml, $P=0.004$). Our results suggested that a suppression of HIV-1 replication by GBV-C co-infection is not mediated by up-regulated RANTES, and thus call for another as yet unknown factor.**

Key words: **GBV-C, HIV-1, RANTES, Co-infection**

GB virus C (GBV-C) is a single-stranded RNA virus belonging to the Flaviviridae family (10, 12, 20). It is distributed worldwide, and five major genotypes that have been recognized are prevalent in various geographical regions (15, 16, 24). GBV-C is transmitted parenterally, and a high prevalence rate of GBV-C infection is observed among hemophiliacs (22.9–38.6%), hemodialysis patients (10.2–16.4%) and intravenous drug users (IVDU) (32.1–34.9%) (2, 4, 17, 23, 27, 28). GBV-C is also transmitted through sexual contacts (5, 11). For example, 12.5% of homosexual men were determined to be infected with GBV-C in our previous report (6).

Due to its close relationship to hepatitis C virus, also a member of the Flaviviridae family, GBV-C was thought to be the cause of hepatitis in humans at the time of discovery in 1995 (12). As it became clear that GBV-C by itself is not a causative agent of hepatitis or any other disease, however, studies shifted toward the influence of GBV-C co-infection.

In 2001, two reports that appeared in the New England Journal of Medicine showed a beneficial effect of the GBV-C co-infection in HIV-1 infection in so far as human immunodeficiency virus type 1 (HIV-1)-infected patients who are co-infected with GBV-C had an improved mortality rate (22, 26). In addition, subsequent studies by Xiang et al. and Jung et al. showed that the co-infection with GBV-C resulted in increased cytokine levels including RANTES (CCL5), SDF-1, and MIP-1, whereby HIV-1 replication is inhibited (7,

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; GBV-C, GB virus C; HAART, highly active antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; IVDU, intravenous drug users; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; RANTES, regulated upon activation, normal T-cell expressed and secreted; RT, reverse transcription; VL, viral load.

25).

In contrast, several other studies have found no beneficial effect of the GBV-C co-infection and one that showed that an increased mortality rate was observed in GBV-C co-infected individuals (8, 18, 19).

We conducted the present study to clarify the complex status of the effect of GBV-C co-infection on HIV-1-infected patients.

Materials and Methods

Patients. HIV-1-infected patients (n=182) who underwent medical examinations at Nagoya Medical Center, Japan, and from whom informed consent was obtained, were enrolled in the study. Of the 182, 151 were male. One hundred forty-three patients were infected with HIV-1 through sexual contacts (78 homosexuals, 53 heterosexuals, and 12 bisexuals). Other routes included via blood or blood products in 13 patients, and IVDU and accidental needle-stick in 1 patient each. One hundred forty-seven patients were Japanese, 19 were from Brazil, 5 from Thailand, 2 each from Peru, America, and Korea, and 1 each from China, Bangladesh, Uganda, Myanmar, and Ukraine.

Samples. Plasma samples of the individuals mentioned above were collected, and stored at −80 C until analyzed. In addition, approximately 1 year after the date of the sample collection, plasma samples were obtained again from the same patients to conduct a follow-up study.

Detection of GBV-C RNA. Purification of RNA from the plasma samples and the reverse transcription-polymerase chain reaction (RT-PCR) method to amplify GBV-C RNA were performed as described in our previous report (6). Briefly, RNA was extracted from the thawed samples using a QIAamp Viral RNA Mini Kit (QIAGEN, Tokyo). The extracted RNA was used in a RT reaction followed by a first-round PCR (RT-PCR) using a Superscript One-Step RT-PCR for long templates (Invitrogen, Tokyo). Subsequently, cDNA was further amplified in a second-round PCR using a TaKaRa LA *Taq* (TaKaRa Shuzo, Tokyo). The same primers and the parameter settings as in our previous report were used for the RT/nested PCR. The PCR products were detected on a 1.2% agarose gel containing ethidium bromide. The expected size of the PCR product was 252 base pairs.

Determination of nucleotide sequences. PCR products of approximately 250 base pairs in size were purified from the agarose gel using a QIAquick Gel Extraction Kit (QIAGEN), and labeled using a BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Tokyo). The nucleotide sequences were determined using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Determination of GBV-C genotypes. The obtained nucleotide sequences were compared against the reference sequences of 5 GBV-C genotypes: genotype 1 (accession #U36380), genotype 2 (U44402), genotype 3 (D90601), genotype 4 (AB013188), and genotype 5 (AF131112) in the GenBank of NCBI. The genotype for each GBV-C-positive sample was determined with an UPGMA method using Genetyx-Mac (version 10.1) system (Software Development, Tokyo).

Quantification of plasma GBV-C load. Total RNA from each GBV-C-positive patient was purified from 200 μl plasma samples using Trizol LS Reagent (Gibco BRL Life Technologies, Md., U.S.A.) according to the manufacturer's instructions. The obtained RNA was dissolved in 10 μl DEPC-treated water, and a half of the volume was reverse transcribed by Superscript II RNase H− Reverse Transcriptase (Invitrogen) at 42 C for 50 min. Subsequently, 2 μl of the 20 μl RT reaction mixture was applied to quantify the GBV-C copy numbers in triplicate by real-time PCR using a TaqMan probe. The same primer/probe set was used as in our previous report (6).

Determination of plasma RANTES concentrations. Concentrations of regulated upon activation, normal T-cell expressed and secreted (RANTES) in the plasma samples were measured using the Human RANTES enzyme-linked immunosorbent assay (ELISA) Kit (Biosource, Calif., U.S.A.) according to the manufacturer's protocol.

Measurement of plasma HIV-1 viral loads and CD4 cell counts. Plasma HIV-1 viral loads were measured using an Amplicor HIV-1 monitor version 1.5 system (Roche, Tokyo). CD4 cell counts were flow-cytometrically measured by FACSCalibur (Becton Dickinson, Tokyo).

Statistical analysis. The means of GBV-C VL among 3 GBV-C genotypes were compared by the Kruskal-Wallis test. The Mann-Whitney U test was used to compare the means of HIV-1 VL, or RANTES concentration between GBV-C co-infected group and uninfected group. Linear regression analysis was used to evaluate the relationship between GBV-C VL and HIV-1 VL. The significance level was set at 0.05, and all P values were two-sided.

Results

Prevalence of GBV-C Infection among HIV-1-Infected Patients

RT/nested PCR was performed to detect GBV-C RNA from the plasma samples of HIV-1-infected

patients. PCR products of approximately 250 bp were detected in 37 of the 182 samples (20.3%) by agarose gel electrophoresis. The characteristics of these patients are summarized in Table 1.

GBV-C Genotypes

Following the determination of the GBV-C prevalence, the nucleotide sequences of the PCR products were analyzed to classify the 37 samples into the 5 GBV-C genotypes. Of the 37 samples, 22 samples were GBV-C genotype 3, which is abundant in Asia. Twelve were classified as genotype 2 found in Europe and the U.S., and the remaining 3 were genotype 1 found in Africa. None were classified as either genotype 4 or 5 (Fig. 1).

Plasma GBV-C RNA Load

The GBV-C copy number in each of the 37 plasma samples was quantified with a real-time PCR method using a Roche LightCycler. Viral loads ranged from 7.8×10^3 to 3.3×10^6 copies/ml. The distribution of the viral loads among GBV-C genotypes 1, 2 and 3 were 7.3×10^5–3.3×10^6 copies/ml (2.4×10^6 copies/ml av.), 7.8×10^3–1.4×10^6 copies/ml (3.4×10^5 copies/ml av.), and 2.1×10^4–2.3×10^6 copies/ml (3.0×10^5 copies/ml av.), respectively (Fig. 2). The average viral loads of the 3 genotypes were compared, and it was found that the viral loads among genotype 1 were significantly higher than for the remaining genotypes ($P=0.04$). However, in the follow-up study, the GBV-C viral loads, which ranged from 2.4×10^1 to 5.1×10^6 copies/ml, did not differ among the 3 genotypes ($P=0.57$) (data not shown). Thus, a difference in viral load among genotypes may not exist.

Table 1. Characteristics of 182 patients with HIV-1 infection, grouped by GBV-C status

	$n=182$	
GBV-C status	+	−
	37	145
Mean CD4+ cell count (cells/ml)	376.8	412.3
HAART received	18	80
Mode of HIV-1 transmission		
Sexual contact	31	112
Homosexual	22	56
Heterosexual	7	46
Bisexual	2	10
Blood or blood products	3	10
IVDU	0	1
Needle-stick	0	1
Unknown	3	21
Sex		
Male	34	117
Female	3	28
Nationality		
Japan	33	114
South America	2	19
East Asia	0	7
South/Southeast Asia	2	1
North America	0	2
Africa	0	1
Easterm Europe	0	1

HAART, highly active antiretroviral therapy; IVDU, intravenous drug use.

HIV-1 VL among HAART-Naïve Patients and Its Correlation with GBV-C VL

To evaluate the effect of GBV-C co-infection on HIV-1-infected patients, we analyzed the HIV-1 viral load of highly active antiretroviral therapy (HAART)-naïve patients. The HIV-1 viral load for 19 GBV-C-infected and 65 GBV-C–uninfected patients ranged from 7.8×10^3 to 3.2×10^6 copies/ml, and from 4.7×10^2 to 1.1×10^6 copies/ml, respectively, and there was a statistically significant difference between the two groups ($P=0.01$) (Fig. 3). In addition, a weakly inverse correlation was observed between the GBV-C viral load and HIV-1 viral load in 19 HAART-naïve GBV-C-co-infected patients (Fig. 4).

Plasma RANTES Concentrations

Plasma RANTES concentrations of the HAART-naïve HIV-1-infected patients with or without GBV-C viremia were measured using a commercially available ELISA kit. The RANTES concentrations of those who showed a GBV-C viremia ranged from 190 to 9,959 pg/ml (3,856 pg/ml av.). Unexpectedly, in those without GBV-C viremia, the RANTES concentration ranged from 264 to 31,038 pg/ml (9,233 pg/ml av.), which was significantly higher than in those with viremia ($P=0.004$) (Fig. 5A).

In the follow-up study, the number of the patients decreased to 47 (11 GBV-C-co-infected, and 36 GBV-C-uninfected patients) since approximately half of the patients had started receiving HAART. The RANTES concentration in those with GBV-C viremia ranged from 72 to 14,007 pg/ml (5,369 pg/ml av.), and in those without viremia ranged from 241 to 22,670 pg/ml (5,973 pg/ml av.). The RANTES levels of the 2 groups were not significantly different ($P=0.86$) (Fig. 5B).

Discussion

We have performed RT/nested PCR to detect GBV-C RNA in the plasma samples of 182 HIV-1-infected patients, who were seen at the Nagoya Medical Center. The prevalence rate was determined to be 20.3%, and

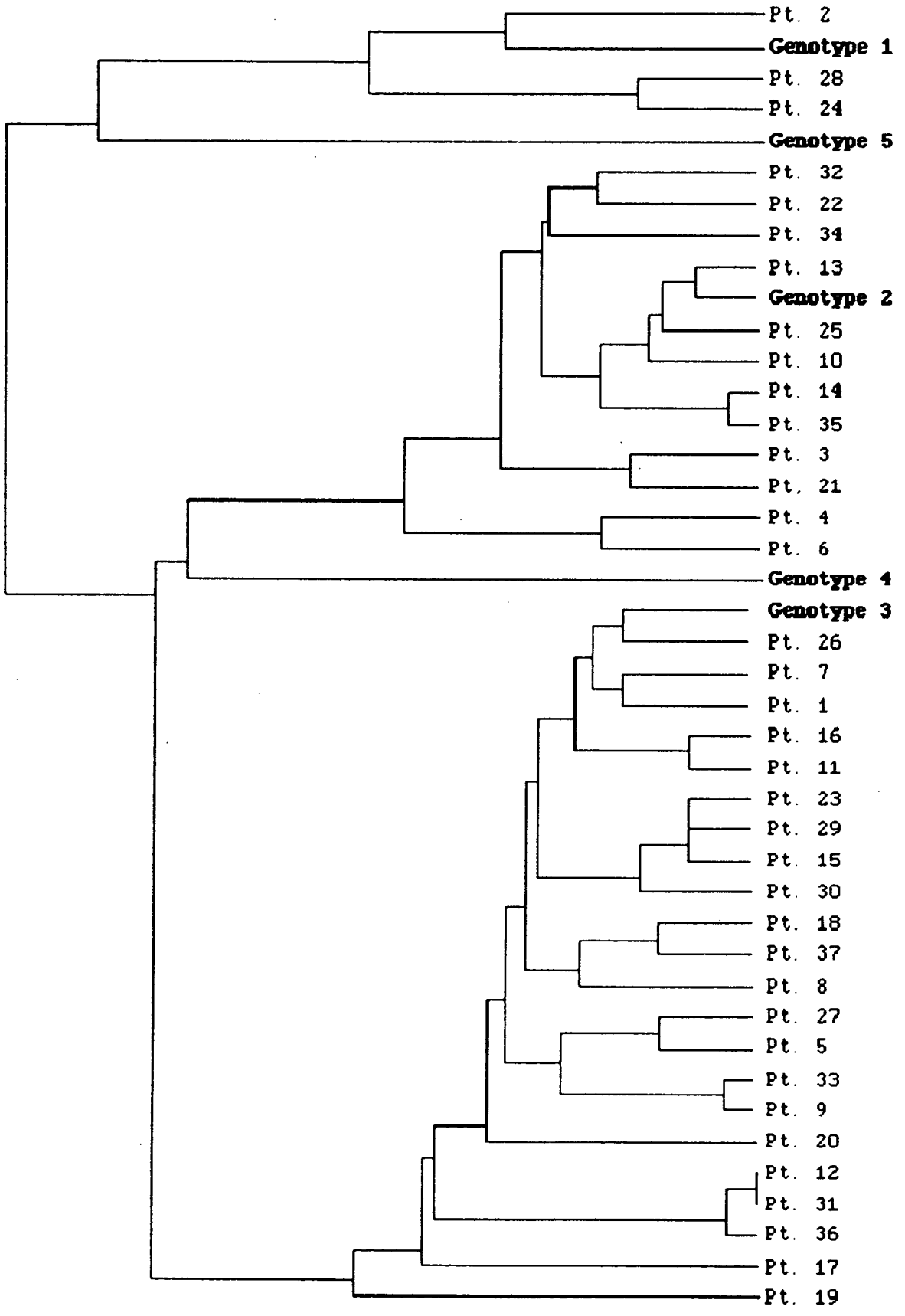

Fig. 1. Phylogenetic tree of GBV-C co-infected patients. The 37 GBV-C-infected patients (Pt.) are numbered 1 through 37. The reference sequences of the 5 GBV-C genotypes are shown in bold letters.

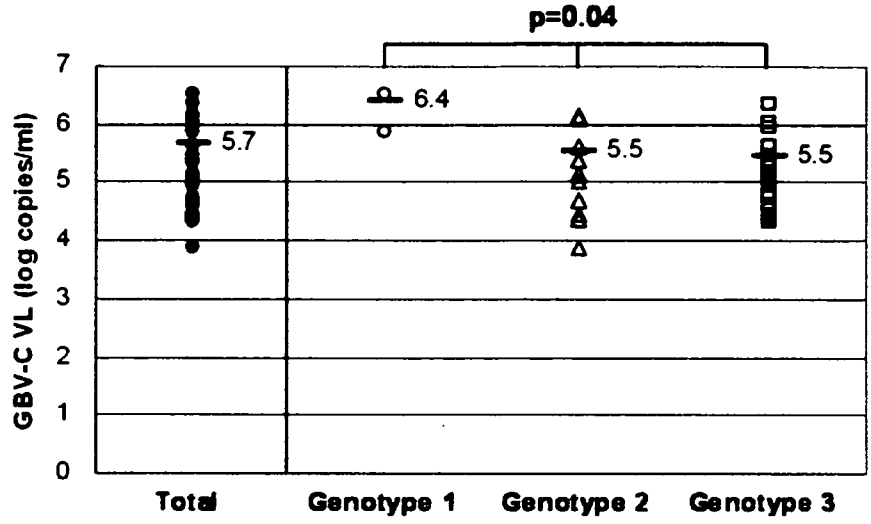

Fig. 2. Distribution of GBV-C viral load among 37 patients (●), and according to the genotypes 1 (○), 2 (△), and 3 (□). The bars indicate the mean viral load.

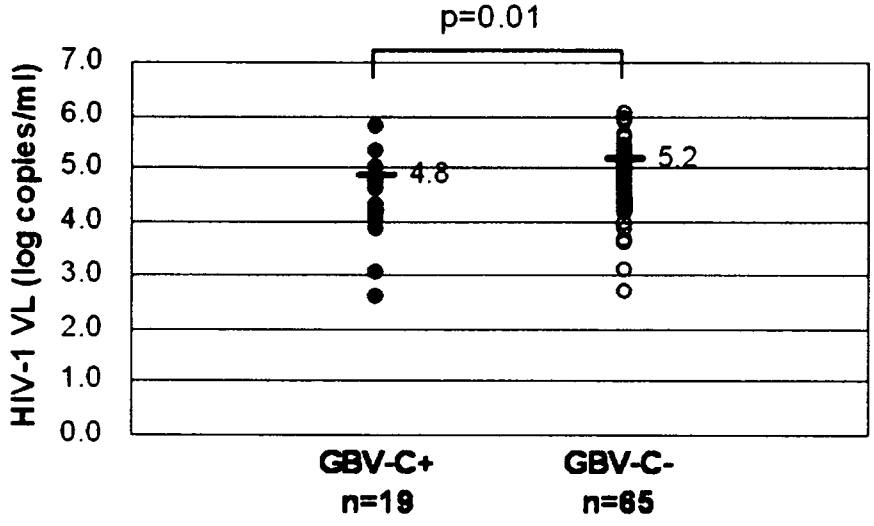

Fig. 3. Distribution of HIV-1 viral load among HAART-naïve GBV-C-co-infected (●) and GBV-C-uninfected (○) patients. The bars indicate the mean viral load.

was similar to the results of HIV-1-infected patients obtained by others (3, 21, 22). This fairly high value seems reasonable as the prevalence seen among homosexual men (12.5%) in our previous report was higher than that of healthy blood donors, and the majority of subjects in this study were infected with HIV-1 through homosexual contacts.

GBV-C genotypic analysis showed that 59.5% (22/37) of the GBV-C-infected individuals were classified as genotype 3, 32.4% (12/37) as genotype 2, and 8.1% (3/37) as genotype 1. None were classified as genotype 4 or 5. Our results agreed with reports by others in that genotype 3 is prevalent in Japan (1, 14). Two of the 3 individuals with GBV-C genotype 1 were hemophiliacs, agreeing with a previous report by Liu et al. (13).

To study the effect of GBV-C co-infection on HIV-1-infected patients, plasma HIV-1 viral loads of antiretroviral therapy-naïve GBV-C-infected and -uninfected groups were compared. Statistical analysis showed that the HIV-1 viral loads of the GBV-C co-infected group were significantly lower than those of the singly-infected group (P=0.01). Consequently, we looked into the relationship between GBV-C viral load and HIV-1 viral

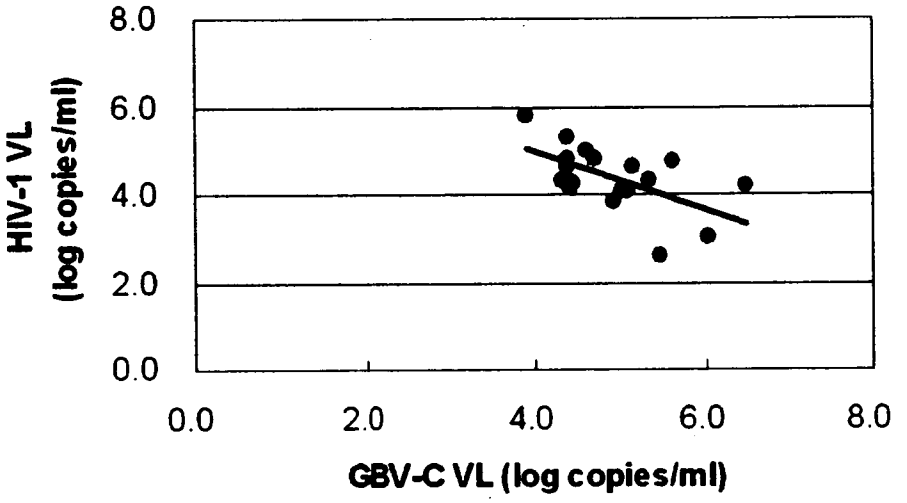

Fig. 4. Correlation between the GBV-C viral load and HIV-1 viral load (R^2=0.34).

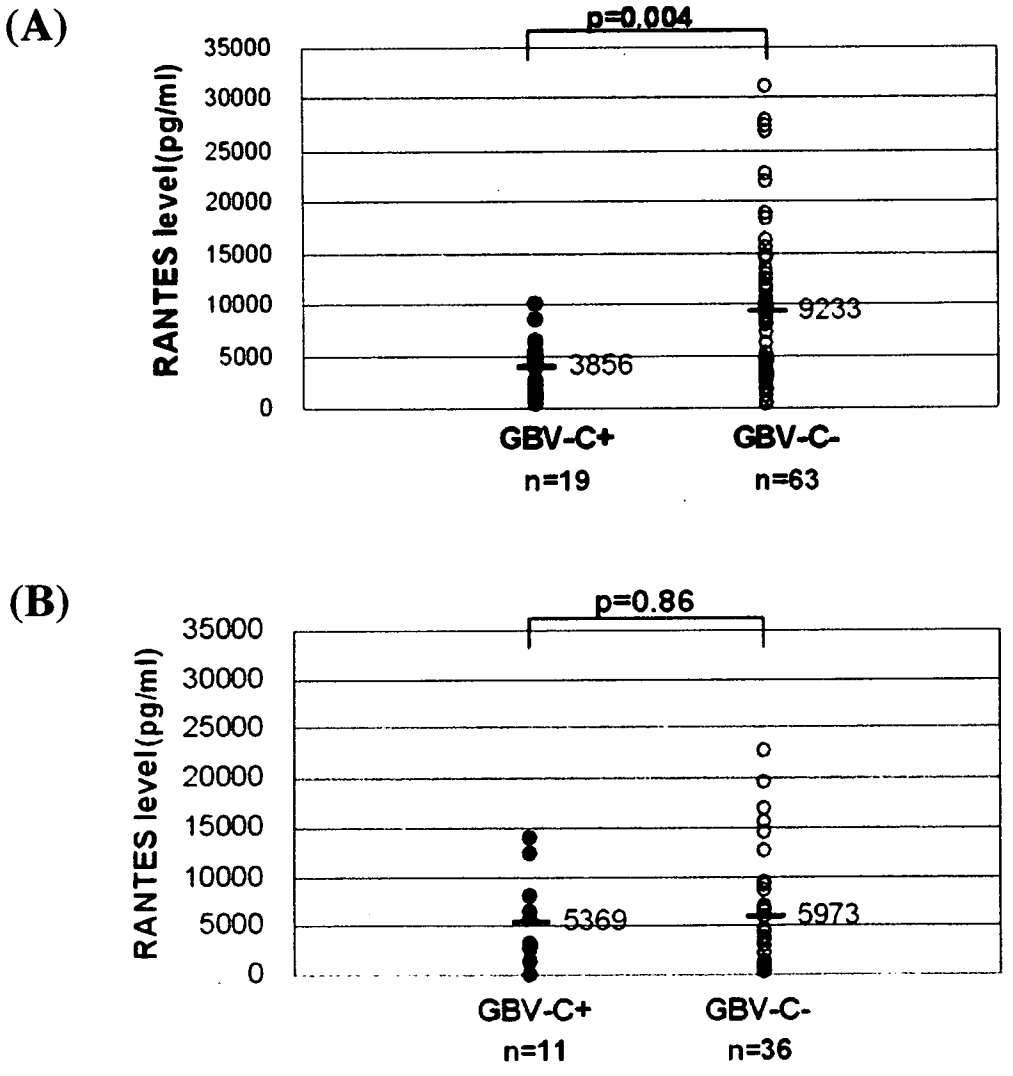

Fig. 5. Distribution of RANTES concentrations among HAART-naïve GBV-C-co-infected (●) and GBV-C-uninfected (○) patients in (A)the initial study, and (B)the follow-up study. The bars represent the mean RANTES concentrations.

load of the same study groups, and observed an inverse correlation in that individuals with higher GBV-C viral loads tended to have lower HIV-1 viral loads (R^2=0.34). Although the suppression level shown here was weak and partial, it is clear that the GBV-C infection is associated with the suppression of HIV-1 replication. Our results do not directly support the findings by Xiang et al. (26) or Tillmann et al. (22), but we believe that the HIV-1 replication suppressive effect by GBV-C possibly leads to a better prognosis and a low mortality rate. The question of why some research groups found a beneficial effect of GBV-C co-infection while others did not still remains. The discrepancy may occur from the differences in race, HIV-1 clade, or GBV-C genotype. However, divergent results were observed even when the studies were composed of mostly Caucasian subjects who were presumably infected with HIV-1 subtype B (18, 19, 22, 26). In addition, the differences in GBV-C genotype do not seem to account for the contrasting findings since the HIV-1 VLs between GBV-C genotypes 2 and 3 were similar as observed in the present study. Thus, other reasons may exist for the conflicting observations.

One possible mechanism of the suppression of HIV-1 replication is the inhibition of HIV-1 entry into target cells. As Xiang et al. reported that the co-infection of GBV-C suppressed the HIV-1 replication through up-regulation of RANTES, a natural CCR5 ligand, and also known as CCL5, in an *in vitro* study (25), we assessed the scenario by measuring the RANTES concentration in patients' plasma. In contrast to the *in vitro* study, however, much higher RANTES concentrations were observed in GBV-C-uninfected individuals. In addition, a mean RANTES level of 3,856 pg/ml in HAART-naïve GBV-C-infected patients at the first medical examination increased to 5,369 pg/ml after 1 year, whereas that of 9,233 pg/ml in HAART-naïve GBV-C-uninfected patients decreased to 5,973 pg/ml. This increase in the RANTES level in the GBV-C co-infected group, and in contrast, the decrease in the uninfected group, was unexpected and is inexplicable. Kwofie et al. reported a transient decrease in RANTES level at the early stage of HIV-1 infection in macaques (9). A similar fluctuation in RANTES level might occur in humans as well. Regrettably, we were unable to conclude that the HIV-1 replication was suppressed by up-regulation of RANTES by GBV-C, and the inhibition of HIV-1 replication might be supported through other mechanisms induced by GBV-C co-infection.

We can conclude that GBV-C co-infection affects the clinical status of HIV-1-infected patients, and that the co-infection confers a beneficial effect on the patient.

References

1) Abe, K. 2001. GB virus-C/hepatitis G virus. Jpn. J. Infect. Dis. **54**: 55–63.
2) Anastassopoulou, C.G., Paraskevis, D., Sypsa, V., Psichogiou, M., Katsoulidou, A., Tassopoulos, N., Skoutelis, A., Malliori, M., and Hatzakis, A. 1998. Prevalence patterns and genotypes of GB virus C/hepatitis G virus among imprisoned intravenous drug users. J. Med. Virol. **56**: 246–252.
3) Antonucci, G., Girardi, E., Cozzi-Lepri, A., Capobianchi, M.R., Morsica, G., Pizzaferri, P., Ladisa, N., Sighinolfi, L., Chiodera, A., Solmone, M., Lalle, E., Ippo, G., Monforte, A., HepaICoNA Study Group, and ICoNA Study Group. 2005. Response to HAART and GB virus type C coinfection in a cohort of antiretroviral-naïve HIV-infected individuals. Antivir. Ther. **10**: 109–117.
4) Christensen, P.B., Fisker, N., Mygind, L.H., Krarup, H.B., Wedderkopp, N., Varming, K., and Georgsen, J. 2003. GB virus C epidemiology in Denmark: different routes of transmission in children and low- and high-risk adults. J. Med. Virol. **70**: 156–162.
5) Frey, S.E., Homan, S.M., Sokol-Anderson, M., Cayco, M.T., Cortorreal, P., Musial, C.E., and Di Bisceglie, A. 2001. Evidence for probable sexual transmission of the hepatitis G virus. Clin. Infect. Dis. **34**: 1033–1038.
6) Hattori, J., Ibe, S., Nagai, H., Wada, K., Morishita, T., Sato, K., Utsumi, M., and Kaneda, T. 2003. Prevalence of infection and genotypes of GBV-C/HGV among homosexual men. Microbiol. Immunol. **47**: 759–763.
7) Jung, S., Knauer, O., Donhauser, N., Eichenmuller, M., Helm, M., Fleckenstein, B., and Reil, H. 2005. Inhibition of HIV strains by GB virus C in cell culture can be mediated by CD4 and CD8 T-lymphocyte derived soluble factors. AIDS **19**: 1267–1272.
8) Kaye, S., Howard, M., Alabi, A., Hansmann, A., Whittle, H., and Schim vander Loeff, M. 2005. No observed effect of GB virus C coinfection on disease progression in a cohort of African women infected with HIV-1 or HIV-2. Clin. Infect. Dis. **40**: 876–878.
9) Kwofie, T.B., Haga, T., Iida, T., Hayami, M., and Miura, T. 2000. Plasma levels of the chemokine RANTES in macaque monkeys infected with pathogenic and non-pathogenic SIV/HIV-1 chimeric viruses at an early stage of infection. J. Vet. Med. Sci. **62**: 1311–1312.
10) Leary, T.P., Muerhoff, A.S., Simons, J.N., Pilot-Matias, T.J., Erker, J.C., Chalmers, M.L., Schlauder, G.G., Dawson, G.J., Desai, S.M., and Mushahwar, I.K. 1996. Sequence and genomic organization of GBV-C: a novel member of the Flaviviridae associated with human non-A-E hepatitis. J. Med. Virol. **48**: 60–67.
11) Lefrere, J.J., Roudot-Thoraval, F., Morand-Joubert, L., Brossard, Y., Parnet-Mathieu, F., Mariotti, M., Agis, F., Rouet, G., Lerable, J., Lefevre, G., Girot, R., and Loiseau, P. 1999. Prevalence of GB virus type C/hepatitis G virus RNA and of anti-E2 in individuals at high or low risk for blood-borne or sexually transmitted viruses: evidence of sexual and parenteral transmission. Transfusion **39**: 83–94.
12) Linnen, J., Wages, J., Jr., Zhang-Keck, Z.Y., Fry, K.E.,

Krawczynski, K.Z., Alter, H., Koonin, E., Gallagher, M., Alter, M., Hadziyannis, S., Karayiannis, P., Fung, K., Nakatsuji, Y., Shih, J.W.K., Young, L., Piatak, M., Jr., Hoover, C., Fernandez, J., Chen, S., Zou, J.C., Morris, T., Hyams, K.C., Ismay, S., Lifson, J.D., Hess, G., Foung, S.K.H., Thomas, H., Bradley, D., Margolis, H., and Kim, J.P. 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science **271**: 505–508.

13) Liu, H.F., Teng, C.W., Fukuda, Y., Nakano, I., Hayashi, K., Takamatsu, J., Goubau, P., and Toyoda, H. 2003. A novel subtype of GB virus C/hepatitis G virus genotype 1 detected uniquely in patients with hemophilia in Japan. J. Med. Virol. **71**: 385–390.
14) Moriyama, M., Zi-Yi, Z., Longren, W., Xueqing, W., Ohshiro, S., Iwasaki, H., Ichijima, S., Iwaguchi, K., Matsumura, H., Sugitani, M., Komiyama, K., and Arakawa, Y. 2001. Clinico-epidemiological study of GBV-C/HGV infection in Tokyo metropolitan, and Nanjing and Yanbian cities in the People's Republic of China. Hepatol. Res. **21**: 268–279.
15) Muerhoff, A.S., Simons, J.N., Leary, T.P., Erker, J.C., Chalmers, M.L., Pilot-Matias, T.J., Dawson, G.J., Desai, S.M., and Mushahwar, I.K. 1996. Sequence heterogeneity within the 5'-terminal region of the hepatitis GB virus C genome and evidence for genotypes. J. Hepatol. **25**: 379–384.
16) Naito, H., Win, K.M., and Abe, K. 1999. Identification of a novel genotype of hepatitis G virus in Southeast Asia. J. Clin. Microbiol. **37**: 1217–1220.
17) Ozdarendeli, A., Toroman, Z.A., Kalkan, A., Kilic, S.S., Ozden, M., and Doymaz, M.Z. 2005. Prevalence and genotypes of hepatitis G virus among hemodialysis patients in Eastern Anatolia, Turkey. Med. Princ. Pract. **14**: 102–106.
18) Quiros-Roldan, E., Maroto, M.C., Torti, C., Moretti, F., Casari, S., Pan, A., and Carosi, G. 2002. No evidence of beneficial effect of GV virus type C infection on the course of HIV infection. AIDS **16**: 1430–1431.
19) Ryt-Hansen, R., Katzenstein, T.L., Gerstoft, J., and Eugen-Olsen, J. 2006. No influence of GB virus C on disease progression in a Danish cohort of HIV-infected men. AIDS Res. Hum. Retroviruses **22**: 496–498.
20) Simons, J.N., Leary, T.P., Dawson, G.J., Pilot-Matias, T.J., Muerhoff, A.S., Schlauder, G.G., Desai, S.M., and Mushahwar, I.K. 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat. Med. **1**: 564–569.
21) Souza, I.E., Zhang, W., Diaz, R.S., Chaloner, K., Klinzman, D., and Stapleton, J.T. 2006. Effect of GB virus C on response to antiretroviral therapy in HIV-infected Brazilians. HIV Med. **7**: 25–31.
22) Tillmann, H.L., Heiken, H., Knapik-Botor, A., Heringlake, S., Ockenga, J., Wilber, J., Goergen, B., Detmer, J., McMorrow, M., Stoll, M., Schmidt, R.E., and Manns, M. 2001. Infection with GB virus C and reduced mortality among HIV-infected patients. N. Engl. J. Med. **345**: 715–724.
23) Toyoda, H., Takahashi, I., Fukuda, Y., Hayakawa, T., and Takamatsu, J. 2000. Comparison of characteristics between patients with GB virus C/hepatitis G virus (GBV-C/HGV) RNA and those with GBV-C/HGV E2-antibody in patients with hemophilia. J. Med. Virol. **60**: 34–38.
24) Tucker, T.J., Smuts, H., Eickhaus, P., Robson, S.C., and Kirsch, R.E. 1999. Molecular characterization of the 5' noncoding region of South African GBV-C/HGV isolates: major deletion and evidence for a fourth genotype. J. Med. Virol. **59**: 52–59.
25) Xiang, J., George, S.L., Wunschmann, S., Chang, Q., Klinzman, D., and Stapleton, J.T. 2004. Inhibition of HIV-1 replication by GB virus C infection through increases in RANTES, MIP-1α, MIP-1β, and SDF-1. Lancet **363**: 2040–2046.
26) Xiang, J., Wunschmann, S., Diekema, D.J., Klinzman, D., Patrick, K.D., George, S.L., and Stapleton, J.T. 2001. Effect of coinfection with GB virus C on survival among patients with HIV infection. N. Engl. J. Med. **345**: 707–714.
27) Yu, M.L., Chuang, W.L., Wang, L.Y., Dai, C.Y., Chiou, S.S., Sung, M.H., Chang, C.S., Shen, S.C., Wang, C.S., Chang, T.T., and Chang, W.Y. 2000. Status and natural course of GB virus C/hepatitis G virus infection among high-risk groups and volunteer blood donors in Taiwan. J. Gastroenterol. Hepatol. **15**: 1404–1410.
28) Zhu, W.F., Yin, L.M., Li, P., Huang, J., and Zhuang, H. 2003. Pathogenicity of GB virus C on virus hepatitis and hemodialysis patients. World J. Gastroenterol. **9**: 1739–1742.

＜症例報告＞

HIV・HCV重複感染に対するリバビリン併用ペグ・インターフェロン療法によりCD4，CD8陽性リンパ球数が減少した1症例

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はじめに

多剤併用療法（highly active antiretroviral therapy：HAART）導入後のHIV感染者の死亡原因は，AIDS関連と非AIDS関連が半々で，非AIDS関連死の約9割が慢性HCV感染症によるといわれている[1]。HIVとHCVの主な感染経路は血液を媒介するもので，静注薬物使用者や血液製剤による感染例で重複感染が多いのが実状である。HIV・HCV重複感染例におけるHCV治療は，HCV単独感染の標準ガイドラインに従いインターフェロンを基本とし，近年ではリバビリンを併用することが多い。

今回，HAART導入によりHIV-RNA量が検出限界（50copies/mL）以下，CD4陽性リンパ球数が200/μL以上と良好なコントロールを得たHIV・HCV重複感染患者に対してリバビリン併用ペグ・インターフェロン療法を行ったところ，CD4，CD8陽性リンパ球数が200/μL以下まで減少した症例を経験したので報告する。

I 症例提示

【症例】49歳，男性

既往歴：血友病B，C型慢性肝炎

臨床経過：1985年にHIV感染が判明し，91年1月よりジドブジン（AZT，レトロビル®）の内服を開始，93年12月からジダノシン（ddI，ヴァイデックス）の内服に変更した。その後，97年4月よりAZT＋ラミブジン（3TC，エピビル®）＋インジナビル（IDV，クリキシバン®）の3剤の内服（HAART）を開始した。2004年9月からAZT＋3TC＋ロピナビル・リトナビル配合剤（LPV/r，カレトラ®）の組み合わせに変更となった。この間，HIV-RNA量は検出限界以下を維持しており，CD4陽性リンパ球数は平均397/μL，CD8陽性リンパ球数は平均910/μLと安定していた。

一方でHCV-RNA量は，04年11月時点で190×10^4IU/mL，05年7月時点で86×10^4IU/mLと高値を示した。そのため，抗HCV療法と

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して，05年8月から06年8月までの1年間，リバビリン併用ペグ・インターフェロン療法を行った。その結果HCV-RNA量は05年10月から陰性となったが，$68\times10^2/\mu$Lあった白血球数は，最も少ない時で$29\times10^2/\mu$Lまで減少した。なかでもCD4陽性リンパ球数は，05年9月で315/μL，12月で194/μL，06年2月で116/μL，4月で134/μL，8月で141/μLと，免疫不全状態の指標である200/μL以下まで減少し，その状態は8カ月にわたり継続した。またCD8陽性リンパ球数も05年9月で656/μL，12月で334/μL，06年2月で171/μL，4月で176/μL，8月で137/μLまで減少した。なおこの間，赤血球数は$23\times10^5/\mu$L以上，血小板数は$9\times10^4/\mu$L以上を維持していたが，ヘモグロビン量については13g/dLから徐々に減少し，06年4月には10g/dL以下となった。そのため，エポエチンアルファ（エスポー®）12000IUの皮下注射を予防投与も含めて4回行い造血の活性化を促した。その効果は残念ながら判然としなかったが，抗HCV療法終了後，赤血球数，ヘモグロビン量および白血球数は増加し，CD4陽性リンパ球数は06年9月で389/μL，10月で291/μLに，CD8陽性リンパ球数もそれぞれ613/μL，442/μLと増加した（**図1**）。

なお抗HCV療法を行うにあたり，リバビリンとAZTの併用による副作用と考えられる貧血を防止するため，HAARTの組み合わせを05年8月からテノホビル（TDF，ビリアード®）＋3TC＋LPV/rに変更した。TDF変更後もHIV-RNA量は検出限界以下を維持していた。

一方でTDFには，副作用として重度の腎機能障害が報告[1]されている。そこでTDF内服開始後のTDF血中濃度（内服3時間後）と血中クレアチニンの推移について検討した。TDF内服前（05年7月）の血中クレアチニンは1.1mg/dLであったが，内服後徐々に上昇し，06年4月以降は1.4mg/dLを超えていた。同様にTDF血中濃度も05年9月での0.45μg/mLから徐々に高くなり，内服1年後の06年9月では0.67μg/mLとなった。通常TDFの最高血中濃度は0.30～0.38μg/mLとされており，本症例では2倍近い高い値を示した（**図2**）。

Ⅱ　考　　察

HIV・HCV重複感染患者に対する抗HCV療法は，HIV-RNA量が良好にコントロールされ，CD4陽性リンパ球数が200/μLを超える安定した患者を対象に行われている[1]。しかしHIV非感染者に比べてHIV・HCV重複感染患者に対しては，インターフェロン投与に伴う白血球数減少やリバビリン投与に伴う貧血などの副作用について特に注意が必要である。なかでもAZTとリバビリンの併用は重度の貧血を起こすことが知られているので[2]，本症例もリバビリン投与開始時にHAARTの組み合わせをAZTからTDFに変更した。またHIV・HCV重複感染患者では，インターフェロンによるCD4陽性リンパ球数の一時的減少が報告[3)～5)]されている。本症例においてもリバビリン併用ペグ・インターフェロン療法を開始した直後から白血球数の減少が認められた。なかでもCD4，CD8陽性リンパ球数は免疫不全状態の指標である200/μL以下まで減少した。本症例ではCD4陽性リンパ球数の減少が緩やかであり日和見感染もみられなかったため，抗HCV療法を継続したが，CD4陽性リンパ球数の減少が重度である場合にはインターフェロンの中止が必要と考える。

一方，リバビリン投与開始時にAZTから変更したTDFについても好中球減少等の副作用が知られており，本症例でもその副作用は顕著に観察された。また本症例では徐々に腎機能が悪化しており，血中クレアチニンの上昇と共にTDF血中濃度も上昇していた。TDFは腎排泄型の薬剤であり，腎機能が悪化すればその代謝も遅延し，血中濃度が上昇すると考えられる。本症例における高いTDF血中濃度と白血球数等の減少との関連は否定できないが，抗HCV療法終了後に再び白血球数が増加

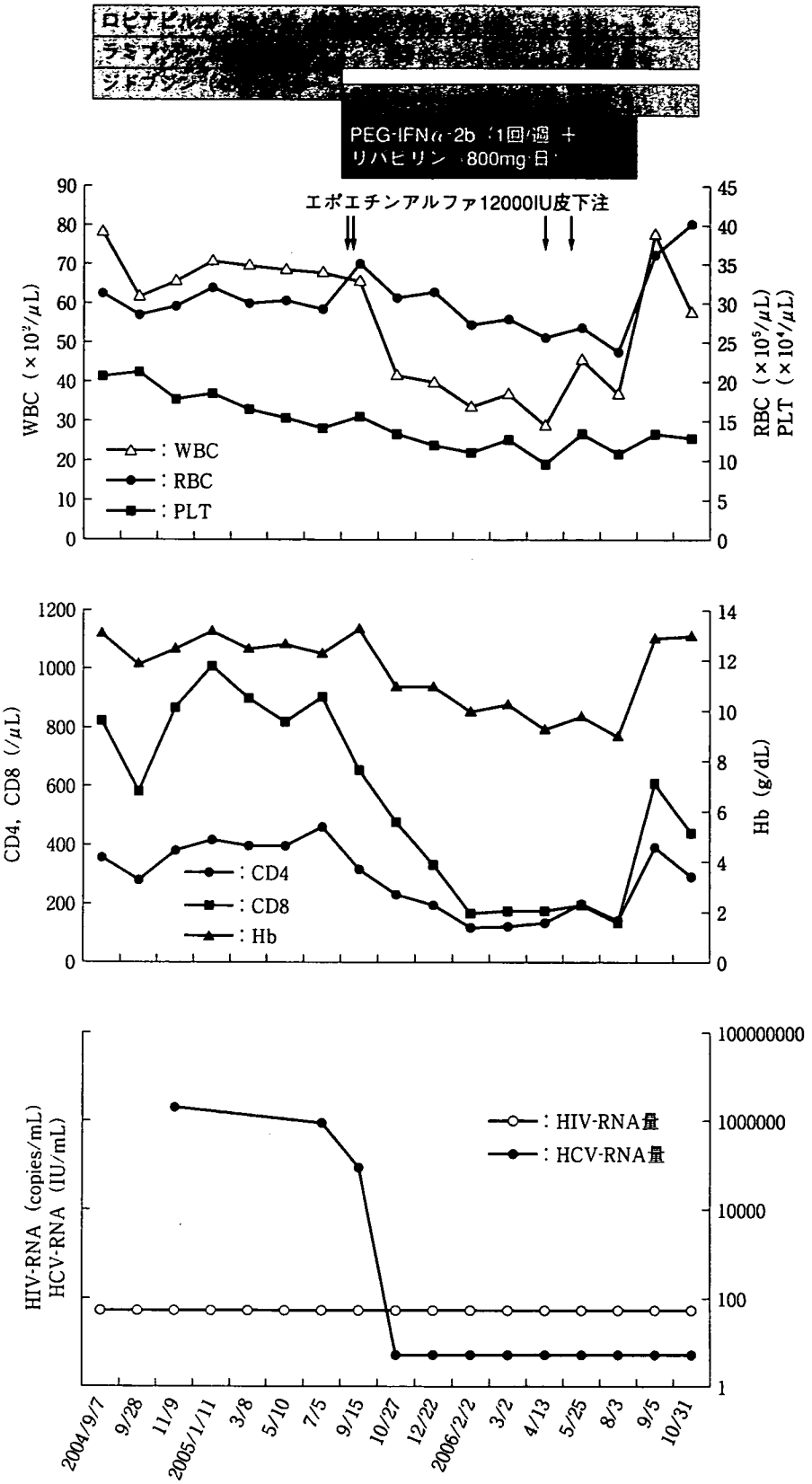
PEG-IFNα-2b 1回/週 +
リバビリン 800mg/日
エポエチンアルファ12000IU皮下注
WBC (×10²/μL)
RBC (×10⁵/μL)
PLT (×10⁴/μL)
: WBC
: RBC
: PLT
CD4, CD8 (/μL)
Hb (g/dL)
: CD4
: CD8
: Hb
HIV-RNA (copies/mL)
HCV-RNA (IU/mL)
: HIV-RNA量
: HCV-RNA量
2004/9/7
9/28
11/9
2005/1/11
3/8
5/10
7/5
9/15
10/27
12/22
2006/2/2
3/2
4/13
5/25
8/3
9/5
10/31

図1　臨床経過

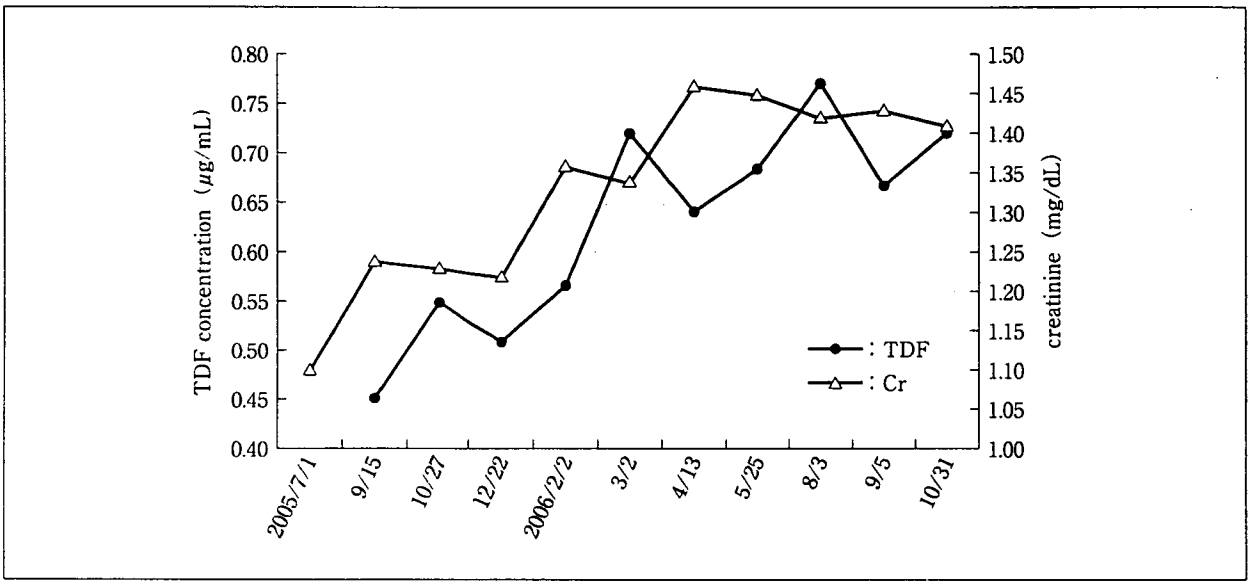

図2　内服3時間後のテノホビル（TDF）血中濃度と血中クレアチニン（Cr）の推移

したことからTDFの関与は小さいと考える。

HIV・HCV重複感染患者に対して抗HCV療法を行う場合には，日和見感染を予防する上でCD4陽性リンパ球数の減少に十分に注意を払う必要がある。加えてHAARTにおいてTDFを選択した場合には腎機能低下に注意し，腎障害時には投与間隔の調節を考慮することが望ましい。

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参　考　文　献

1）HIV感染症治療研究会：HIV感染症「治療の手引き」第10版，p.26，HIV感染症治療研究会事務局，東京，2006.

2）小池和彦：AIDS/HIV-1感染症とウィルス肝炎，医学のあゆみ　**213**，886～890，2005.

3）Laguno, M., Murillas, J. et al.：Peginterferon alfa-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for treatment of HIV/HCV co-infected patients, AIDS　**18**, F27～36, 2004.

4）立浪　忍，瀧　正志：HIV感染血友病におけるHCV感染について，日本エイズ学会誌　**8**，7～10，2005.

5）Santin, M., Shaw, E. et al.：Efficacy and safety of pegylated interferon-α2b plus ribavirin for the treatment of chronic hepatitis C in HIV-infected patients, AIDS Res. Hum. Retroviruses **22**, 315～320, 2006.

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Original Article

Performance and Quality Assurance of Genotypic Drug-Resistance Testing for Human Immunodeficiency Virus Type 1 in Japan

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SUMMARY: Highly active antiretroviral therapy (HAART) can suppress human immunodeficiency virus type 1 (HIV-1) replication and plasma HIV-1 to below detectable levels. However, HAART becomes ineffective when drug-resistant viruses emerge during HAART. Monitoring drug-resistance mutations in viruses is necessary for selecting new drugs or therapies effective at inhibiting such HIV-1 variants. Most laboratories in Japan perform the tests using in-house protocols. However, the quality of these tests has never been assessed. Our study assessing the accuracy and reliability of HIV-1 genotypic drug-resistance testing in 15 laboratories in Japan revealed that the quality was very high (97.3% accurate). The errors, though rare, were caused by human errors, poor electropherograms, and the use of inadequate primers. Here, we propose troubleshooting procedures to improve testing accuracy and reliability in Japan.

INTRODUCTION

Genotypic drug-resistance testing for human immunodeficiency virus type 1 (HIV-1) is clinically useful for successful antiretroviral treatment (1-5). In Japan, the test, which could initially be performed at only a few virological laboratories in 1996, is now conducted at more than 15 laboratories. The cost of testing is almost entirely covered by government research funds. The laboratories use in-house protocols, the main advantage of which is their low cost relative to that of commercial kits. As the quality of these protocols had not previously been evaluated, we conducted an assessment of in-house protocol reliability by sending HIV-1 RNA samples to the relevant laboratories.

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MATERIALS AND METHODS

Laboratories participating in the quality assurance assessment of genotypic drug-resistance testing protocols: Eight HIV/AIDS clinical centers, five local government institutes of public health, one commercial laboratory, and the National Institute of Infectious Diseases (NIID) were enrolled in this study on voluntary basis.

Construction of drug-resistant HIV-1 clones: We chose two plasma specimens, i.e., viruses well-characterized for harboring multidrug-resistance mutations. The HIV-1 of one specimen (case 1) had eight drug-resistance mutations: L63P, A71T, and V77I in the protease (PR), and A62V, V75I, F77L, F116Y, and Q151M in the reverse transcriptase (RT); and 10 mutations unrelated to drug-resistance: V3I, E35D, S37N, R41K, and K70R in the PR, and S68G, T69V, E122K, Q197E, and R211K in the RT. The HIV-1 of the other specimen (case 2) had nine drug-resistance mutations: L10I, L63P, V77I, and L90M in the PR, and M41L, T69S-SG insertion, G190A, L210W, and T215Y in the RT; and 15 mutations unrelated to drug-resistance: V3I, I15V, S37D, and I93L in the PR, and

V35T, T39A, K43E, E122K, I135T, R172K, D177E, Q207H, R211K, L214F, and K238S in RT.

HIV-1 RNA was extracted from the plasma using the QIAamp Viral RNA kit (QIAGEN, Valencia, Calif., USA). DNA fragments sized 1.3 kb containing *gag* (codons #412-500), *protease* (codons #1-99), and *reverse transcriptase* (codons #1-260) were amplified by RT-PCR using SuperScript One-Step RT-PCR (Invitrogen, Carlsbad, Calif., USA) and the primer pair K1 (5′-AAG GGC TGT TGG AAA TGT GG-3′) and U13 (5′-CCC ACT CAG GAA TCC AGG T-3′), followed by a second-round PCR using LA Taq (Takara, Shiga, Japan) with the primer pair INF-ApaI (5′-TGC TGG GCC CCT AGG AAA AAG G-3′) and INF-NheI (5′-TCT GGC TAG CCC AAT TCA ATT TTC CCA C-3′). The amplified fragments were sequenced and the presence of the target mutations was confirmed. The amplicon was then digested with *Apa*I (Takara) and *Nhe*I (New England Biolabs, Ipswich, Mass., USA), and the *Apa*I-*Nhe*I fragment was inserted into the corresponding site of a pSUM9 HXB2 expression vector (kindly provided by Dr. H. Mitsuya, National Cancer Institute, Bethesda, Md., USA) (6). Each clone was transfected into MT-2 cells (approximately 40% confluence in 1 ml of RPMI 1640) with Lipofectamine (Invitrogen). The cells were cultured with RPMI 1640 containing 10% FBS under a 5% CO_2 atmosphere at 37°C. After 4 days, 0.2 ml of the culture supernatant was collected and transferred to 5×10^4 MT-4 cells in 4.8 ml of culture medium. Three days after infection, the culture supernatants were harvested and stored at −80°C until use.

Preparation and shipping of HIV-1 RNA samples: HIV-1 virions were precipitated by ultracentrifugation at 23,000 $\times g$ for 1 h at 4°C and washed twice with PBS(−). The pellets were suspended in 100 μl of PBS(−) and incubated with 3 U of deoxyribonuclease (RT Grade) (Nippon Gene, Tokyo, Japan) at 37°C for 15 min. Virions were precipitated again by ultracentrifugation at 23,000 $\times g$ for 1 h at 4°C and washed twice with PBS(−) to remove the deoxyribonuclease. RNA was extracted using the QIAamp Viral RNA kit. The number of HIV copies was determined by real-time PCR according to the method reported by Nagai et al. (7), and aliquots containing 2×10^4 copies of HIV-1 RNA in 2 ml of PBS(−) were stored at −80°C. The samples were packed on dry ice and shipped to the participating laboratories.

Data collection and evaluation: Each laboratory was requested to provide a detailed protocol for genotypic testing, including the primer sequences and the enzymes used for reverse transcription and PCR conditions. Laboratories were asked to submit electropherograms and a list of drug-resistance mutations determined according to International AIDS Society-USA panel criteria, version March/April 2005 (8).

The rate of successful detection of mutations was expressed as follows: %DR (or NDR) = detected number of drug-resistance mutations (non-drug-resistance mutations)/total number of drug-resistance mutations (non-drug-resistance mutations). Reported mutations absent in the test samples (ghost mutations) were noted as "E1" errors, erroneous categorization as an "E2" error, and errors in preparing the reports as "E3" errors.

RESULTS

Variations in protocols for genotypic drug-resistance testing in 15 laboratories: As shown in Figure 1, the enrolled laboratories used different protocols with respect to primers, sizes of amplified fragments, and the number of amplified fragments. Eight laboratories (A, B, D, E, F, I, J, and K) used the latest NIID protocol with minor modifications, and three laboratories (G, H, and N) used the 1996 version of the NIID protocol with or without modifications. Four laboratories (C, L, M, and O) used their own protocols.

Regarding the reverse transcription and amplification procedures, 12 laboratories used one-step RT-PCR, and the other three laboratories used two-step RT-PCR. In the reverse-transcription process, AMV RT was used in two laboratories, and M-MLV RT was used in one laboratory. As regards the DNA polymerase used for the first and second PCR, most laboratories used Taq polymerase but other few laboratories used KOD polymerase.

For sequencing, Big Dye Terminator V1.1 (Applied Biosystems, Foster City, Calif., USA), Big Dye Terminator V3.1 (Applied Biosystems), CEQ Dye Terminator Cycle Sequencing with a Quick Start kit (Beckman Coulter, Fullerton, Calif., USA), and Thermo Sequenase Cycle Sequencing Kit (USB, Cleveland, Ohio, USA) with IRDye™ 800 v2 Terminator Mixes (LI-COR, Lincoln, Nebr., USA) were used in eight, five, one, and one of the laboratories, respectively. For the purification of labeled products, CENTRI SEP Spin Columns (Applied Biosystems), Sephadex G-50 (GE Healthcare Bio-Sciences, Piscataway, N.J., USA), and DyeEx 2.0 Spin kit (QIAGEN) were used in five, three, and one of the laboratories, respectively. The other six laboratories used the ethanol precipitation method.

For electrophoresis, 14 laboratories used a capillary-type auto-sequencer and the remaining laboratory used a plate-type auto-sequencer. Ten laboratories used the ABI PRISM 310 (Applied Biosystems) auto-sequencer and five laboratories used other sequencers, i.e., the CEQ 8000 (Beckman Coulter), LI-COR 4200 IR2 System (LI-COR), ABI PRISM 3100 (Applied Biosystems), ABI PRISM 3100 Avant (Applied Biosystems), and ABI PRISM 3730S (Applied Biosystems) auto-sequencers.

Results of case 1 testing: As shown in Table 1, in the PR mutation analyses, all laboratories except G detected 100% of the drug-resistance mutations. On the other hand, when detecting the non-drug-resistance mutations, the amino acid mutations reported by laboratories G and H were totally different from those reported by the other laboratories. Laboratory G missed one drug-resistance mutation, A71T, and two non-drug-resistance mutations, E35D and R41K. Laboratory H reported all of the three drug-resistance mutations correctly, but not all of the five non-drug-resistance mutations. Laboratories G and H appear to have amplified the wrong samples, suggesting that contamination may have occurred. Checking their protocols, we noticed that laboratories G and H used DRPRO3 primer in their second round of PCR (Figure 1). As the case 1 sample had an insertion mutation in the DRPRO3 annealing region, a mismatch with this primer in the region caused these errors.

Laboratories E and F reported ghost mutations D29N and C95W, respectively. In the case of laboratory E, the error appeared to be due to the high background noise in the electropherogram. In the case of laboratory F, the data were analyzed only by automatic base sequence analysis, and no manual editing was performed to eliminate the error.

Two laboratories (A and B) made errors in their final reports. We confirmed the raw data from these two laboratories, and found that their electropherograms, nucleotide

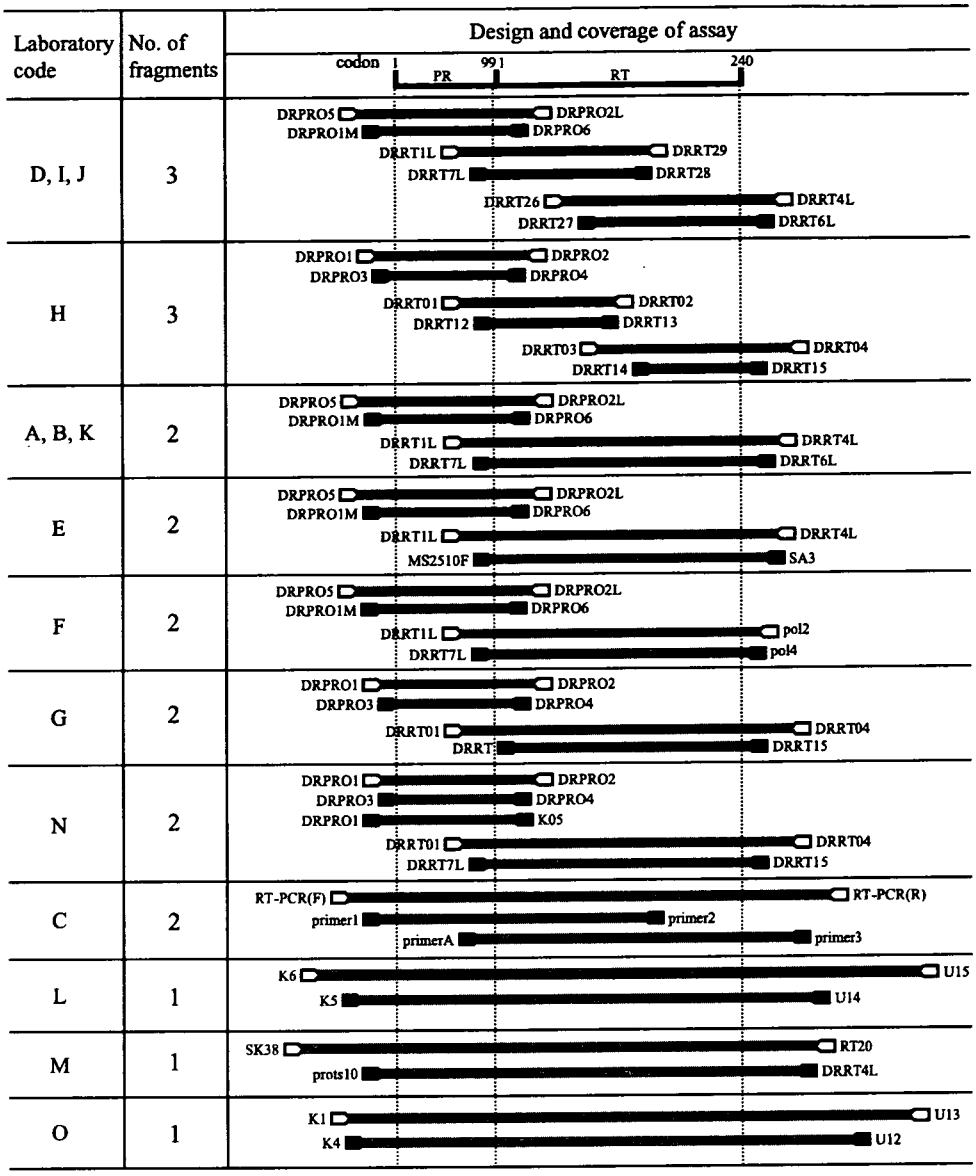

Fig. 1. Schematic pictures showing primer-binding positions on HIV-1 templates and their amplified regions. Primers for RT-PCR and nested PCR are indicated by open and closed symbols, respectively. The letters A to O indicate the 15 laboratories.

sequences, and amino acid sequences were correct, but that they misrecorded the amino acid mutations during the preparation of their final reports.

As regards the RT results, 14 laboratories correctly reported all of the drug-resistance and non-drug-resistance mutations. Laboratory C did not report three non-drug-resistance mutations (S68G, Q197E, and R211K), although these were correctly displayed in the nucleotide and amino acid sequence files, thus indicating an error in the preparation of the final report.

Results of case 2 testing: In the PR mutation analysis, 12 laboratories correctly reported all of the drug-resistance and non-drug-resistance mutations. Laboratory D failed to report the L90M drug-resistance mutation owing to a translation error, although "ATG" was clearly seen in the electropherogram. Laboratory E reported ghost mutation E34K due to the high background noise in the electropherogram. Peak distortion on the electropherograms caused Laboratory F to report ghost mutations R41K and T96S, and an extra large peak on the electropherogram caused the misdetection of L10L/S instead of L10I. This extra large peak may be due to the insufficient removal of residual free dideoxynucleotides after the sequence reaction.

As regards the RT region, 14 laboratories, but not laboratory K, correctly reported all drug-resistance mutations. Laboratory K detected the T69S-SG insertion, but misidentified it as a non-drug-resistance mutation. As for the non-drug-resistance mutations, four laboratories (G, H, L, and N) prepared incorrect reports. Laboratory G did not report V35T and T39A because they were unable to analyze the region between codon #1 and #39. Laboratory H did not report E122K and I135T, because they did not amplify the RT region from codon #122 to #135. These errors reported from laboratories G and H were excluded in the calculation of the rate of detection of non-drug-resistance mutations (%NDR). Laboratory L failed to report K238S, although it was detected in the amino acid sequence file. Laboratory N reported ghost mutation I31T.

DISCUSSION

To assess the quality of in-house genotypic drug-resistance testing, we used two HIV-1 RNA specimens to conduct profi-