Table 2 eGFR recovery rate and duration of TDF administration in TDF discontinuation patients

	No. of patients (%)	Median (interquartile range)	
		eGFR at TDF discontinuation (mL/min/1.73 m^2)	Duration of TDF administration (days)
(1) Recovery group			
Patients with 100% recovery of eGFR after TDF discontinuation	9 (42.9%)	48.1 (46.6–57.7)	28 (6–941)
(2) Mild recovery group			
Patients with mild recovery of eGFR after TDF discontinuation	7 (33.3%)	48.2 (41.4–51.7)	405 (250–1,379)
(3) Exacerbation group			
Patients with exacerbation of eGFR after TDF discontinuation	5 (23.8%)	49.2 (48.3–52.3)	1,110 (421–1,470)

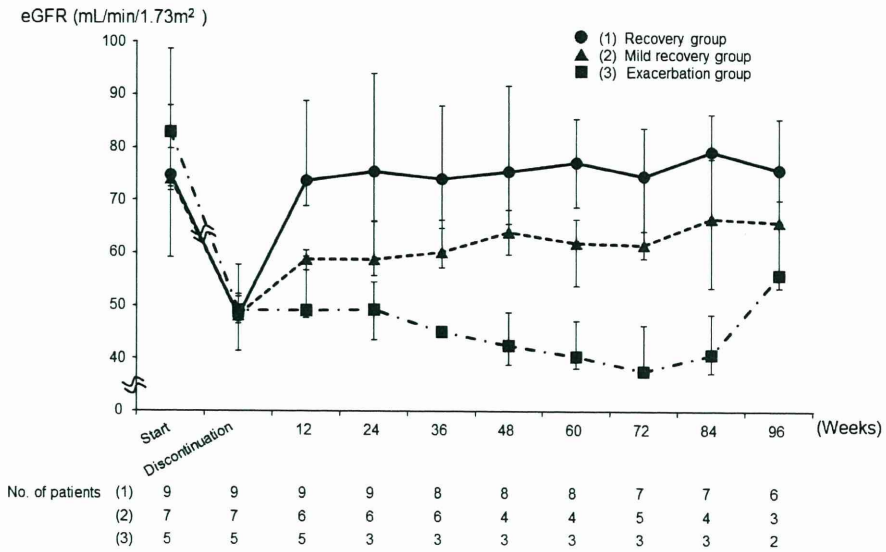

Fig. 2 Changes in eGFR (median ± interquartile range) in each eGFR recovery rate group of TDF discontinuation patients. TDF discontinuation patients were divided into a recovery group (9 patients), mild recovery group (7 patients), and exacerbation group (5 patients), and the changes in eGFR (median ± interquartile range) after discontinuation are shown for each group

followed by ganciclovir and fluconazole ($n = 4$ each), rifabutin, pentamidine, sulfamethoxazole, and azithromycin ($n = 2$ each), and isoniazid, ethambutol, amikacin, and clarithromycin ($n = 1$ each). Most patients received combination therapy with 2–4 of these drugs. In AIDS patients, there is a possibility of deceased renal function both from AIDS itself and from drugs used to treat opportunistic infections; thus, sufficient caution is needed in these patients.

The renal impairment caused by TDF is thought to be reversible with the discontinuation of TDF [9–12], but in investigations using eGFR, there are patients in whom a full recovery is not seen following the discontinuation of TDF [13, 14]. In the present results, eGFR recovered rapidly for 12 weeks after discontinuation, and significant recovery was seen until 96 weeks. However, examining individual cases revealed that some patients exhibited only a mild recovery of eGFR after the discontinuation of TDF, or even exacerbated decreases in eGFR from the level at the time of discontinuation. Recovery of eGFR to the level at the start of TDF was seen in 42.9% of patients, similar to the 42% reported by Wever et al. [14]. The fact that patients are occasionally seen in whom renal function does not recover even after discontinuation suggests the possibility that impairment of renal function may be irreversible after the discontinuation of TDF, depending on the patient.

To investigate the possible factors related to this irreversible impairment of renal function, we classified the trends in eGFR following TDF discontinuation into a recovery group, a mild recovery group, and an exacerbation group, and investigated the effect of duration of TDF administration. The recovery of eGFR was quicker with shorter durations of TDF administration. Full recovery was not seen in patients who received TDF over a long period and in whom renal function declined gradually. In 5 of 9 patients in the recovery group, renal function declined rapidly within 1 month of the start of TDF administration, and in each of these 5 cases, eGFR recovered quickly after discontinuation to the level at the start of administration.

In the mild recovery and exacerbation groups, however, none of the patients showed a rapid decline in renal function within 1 month. The decline was gradual over a long period in nearly all of these patients, and insufficient recovery or an exacerbation in eGFR was seen after discontinuation. Therefore, in patients who receive TDF over a long period, a state of gradual decline in renal function continues with accumulating damage to renal tubules from TDF, which is a likely factor in the irreversibility of damage to renal function after the discontinuation of TDF. The analysis performed in this study was done with a limited number of patients, so the factors related to irreversible damage to renal function after discontinuation of TDF could not be fully clarified. However, a correlation was confirmed between the duration of TDF administration and the eGFR recovery rate.

In recent years, Japanese and other Asians, who have a small build, have been reported to be susceptible to renal impairment caused by TDF [15, 16]. We investigated whether the recovery of renal function after the discontinuation of TDF varied depending on the eGFR equation used. No differences were observed between the eGFR equation for Japanese individuals (2008 Japanese Society of Nephrology calculation) and the eGFR equation that used a Cockcroft–Gault (CG) equation that considered the effects of body weight, and a similar trend was seen in the eGFR recovery rate after the discontinuation of TDF. On the other hand, body weight data could not be obtained at all measurement points during follow-up in the present study, and an analysis of eGFR using the CG equation and an investigation of the effects of body weight therefore could not be sufficiently conducted. In the future, it may be necessary to investigate relationships to body weight and body surface area, as well as the effects of TDF blood concentrations, in Japanese patients, who have a small build, and AIDS patients, who have a tendency to lose weight.

Our findings suggest that when discontinuation criteria for TDF are established based on sCr or eGFR, renal function may not recover following the discontinuation of TDF, particularly among patients who have received long-term administration of TDF and exhibit a gradual decline in renal function. Urinary β_2-microglobulin and tubular reabsorption of phosphate have been reported to be useful markers for detecting renal tubular dysfunction caused by TDF at an earlier stage [13]. Because long-term administration of TDF is expected to continue in the future, it is important to investigate the use of markers that enable earlier and more sensitive detection of renal impairment caused by TDF to complement the assessment of renal function using eGFR.

With advances in treatment, HIV infection has gone from being a fatal condition to being a chronic disease that can be managed medically. At the same time, the various side effects that accompany long-term medication are becoming clear, and measures to improve the long-term prognosis of HIV-infected patients will be an issue in the coming years. In the guidelines of the United States Department of Health and Human Services, revised January 10, 2011, the first recommendation as an NRTI is TDF/emtricitabine (FTC) alone [1]. Thus, the number of patients who continue ART including long-term TDF administration is predicted to increase, and management of renal function will be important. It is reported that approximately 30% of patients with HIV infection have pre-existing renal abnormalities, and factors in renal disease are reported to be adverse effects from anti-HIV drugs or agents to treat opportunistic infection and complications such as HIV-associated nephritis, diabetes mellitus, and hypertension [17, 18]. Given the possibility that the duration of TDF administration affects recovery from renal impairment caused by TDF, it is important when using TDF to consider the increased risk factors for concomitant diseases such as diabetes and hypertension with aging of the patient, in addition to avoiding renal impairment from drugs, such as concurrent medications. Moreover, when renal function declines gradually in patients who receive long-term administration of TDF, sufficient care must be exercised in the management of renal function and in attempts to improve the long-term outcome.

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Outbreak of Infections by Hepatitis B Virus Genotype A and Transmission of Genetic Drug Resistance in Patients Coinfected with HIV-1 in Japan[▽]

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The major routes of hepatitis B virus (HBV) infection in Japan has been mother-to-child transmission (MTCT) and blood transfusion. However, HBV cases transmitted through sexual contact are increasing, especially among HIV-1-seropositive patients. To understand the molecular epidemiology of HBV in HBV/HIV-1 coinfection, we analyzed HBV genotypes and HIV-1 subtypes in HBV/HIV-1-coinfected patients at Nagoya Medical Center from 2003 to 2007. Among 394 HIV-1-infected Japanese men having sex with men (MSM) who were newly diagnosed during the study period, 31 (7.9%) tested positive for the hepatitis B virus surface antigen. HBV sequence analyses were successful in 26 cases, with 21 (80.7%) and 5 (19.3%) cases determined as genotypes A and C, respectively. Our finding that HBV genotype A was dominant in HIV-1-seropositive patients alerts clinicians to an alternative outbreak of HBV genotype A in the HIV-1-infected MSM population and a shift in HBV genotype from C to A in Japan. The narrow genetic diversity in genotype A cases suggests that genotype A has been recently introduced into the MSM population and that sexual contacts among MSM were more active than speculated from HIV-1 tree analyses. In addition, we found a lamivudine resistance mutation in one naïve case, suggesting a risk of drug-resistant HBV transmission. As genotype A infection has a higher risk than infection with other genotypes for individuals to become HBV carriers, prevention programs are urgently needed for the target population.

The number of hepatitis B virus (HBV)-infected persons in Japan is estimated to be 1 million, or 0.8% of the total population (31). HBV is classified into eight genotypes, A to H, by their differences in genome sequences (11, 12, 22). Circulating genotypes in Japan differ according to geographical region, with the prevalent genotypes in 2001 being C (84.7%) and B (12.2%), while A (1.7%) and D (0.4%) were less frequent (17). HBV infection in Japan has been transmitted mainly by two routes, mother-to-child transmission (MTCT) and blood transfusion, which have been targeted by prevention programs still being operated today (13, 15–17, 25).

Regarding MTCT, all pregnant women are screened for HBV antigen and antibody. Mothers who are HBV infected are prohibited from breast-feeding, and their newborns are vaccinated against HBV. Regarding infection by blood transfusion, all donated blood is tested by anti-hepatitis B surface antibody (HBsAb) testing and PCR to exclude HBV-contaminated blood from the supply. These prevention programs have been successful, and the risks of HBV infection by these two routes have been reduced dramatically.

However, HBV infection by sexual contact has recently become a prevailing alternative transmission route of HBV in Japan (30, 36). In particular, coinfection with HBV and human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, has been increasing among men who have sex with men (MSM), and the incidence of HBV infection associated with HIV-1-seropositive cases appeared to be 8.8%, which is higher than that in the general population (5). Thus, the epidemiology of HBV infection in Japan is quickly shifting. Here we report the most recent molecular epidemiologic status of HBV/HIV-1 coinfection.

MATERIALS AND METHODS

Sample. HIV/AIDS patients newly diagnosed at Nagoya Medical Center from 2003 to 2007 were tested for hepatitis B surface antigen (HBsAg), and HBsAg-positive patients were enrolled in the study. Clinical data (age, gender, suspected route of HIV-1 infection, aspartate aminotransferase [AST] and alanine aminotransferase [ALT] plasma levels, CD4-positive T cell count, and HIV viral load) were obtained from medical records. Plasma HBV viral load was measured with COBAS TaqMan (Roche Diagnostics, Basel, Switzerland), and plasma HBc IgM titer was measured with Lumipulse (Fujirebio, Tokyo, Japan). The time of HBV infection was estimated by patient interview and HBc IgM titer results. This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Institutional Review Boards of the National Institute of Infectious Diseases and Nagoya Medical Center. All pa-

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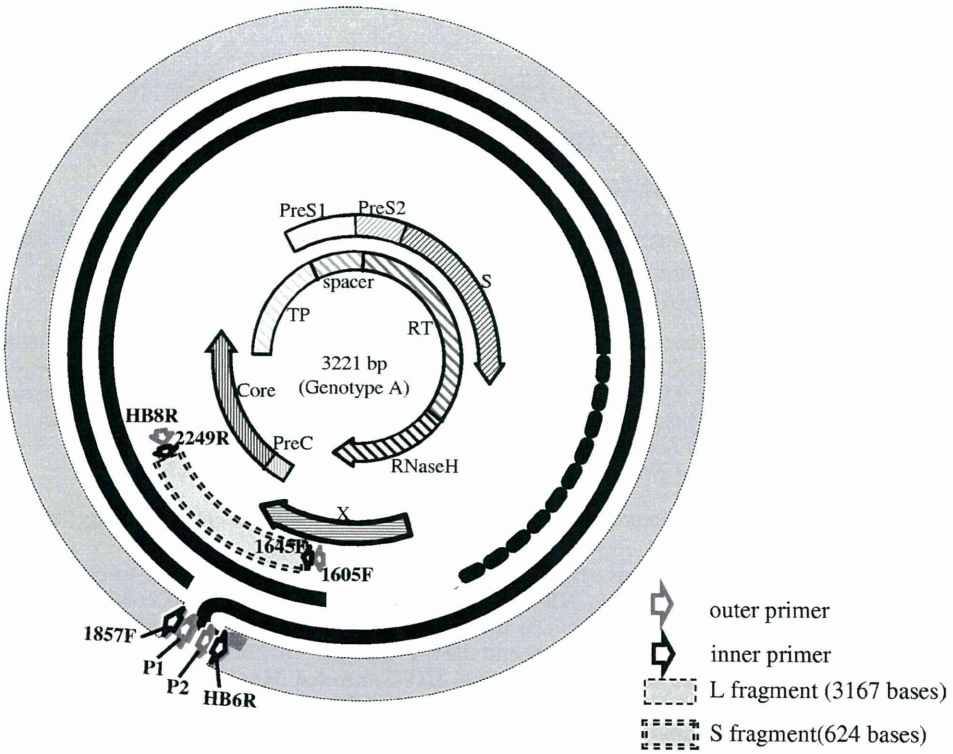

FIG. 1. Genetic regions of HBV and HIV-1 used for phylogenetic tree analyses. The whole HBV genome was amplified in two fragments, L and S, and assembled. L and S fragments are indicated by single and double dashed lines, respectively.

tients provided written informed consent for collection of samples and subsequent analysis.

Amplification of HBV and HIV DNA fragments and determination of DNA sequences. HBV nucleic acid was extracted from plasma using a MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics). As shown in Fig. 1, the full-length HBV genome was amplified in two fragments, L (3,167 bases) and S (624 bases). The primers used for amplifying HBV DNA were both newly designed and have been published previously (27). Details of these primers are summarized in Table 1. The DNA polymerases used for the first and nested PCRs were LA *Taq* (Takara, Shiga, Japan) and Prime Star HS (Takara) polymerase, respectively. The HBV genotypes were also determined using a commercial kit (Institute of Immunology, Tokyo, Japan) based on enzyme immunoassay to confirm that the results did not differ from those based on phylogenetic tree analysis.

The HIV-1 *gag p17* (396 bp [bp 790 to 1185]), *pol* (1,117 bp [bp 2253 to 3369]), and *env C2V3* (222 bp [bp 6996 to 7217]) regions were amplified from extracted plasma HIV-1 RNA by reverse transcription-PCR (RT-PCR) using the SuperScript one-step RT-PCR system for long templates (Invitrogen, Carlsbad, CA)

TABLE 1. Primers for amplifying the HBV and HIV-1 genomes

Name	Direction[a]	Sequence (5′ → 3′)	Region
P1	F	TTTTCACCTCTGCCTAATCA	First PCR, HBV L fragment
P2	R	AAAAAGTTGCATGGTGCTGG	First PCR, HBV L fragment
1605F	F	CGCATGGAGACCACCGTGAA	First PCR, HBV S fragment
HB8R	R	ATAGGGGCATTTGGTGGTCT	First PCR, HBV S fragment
1857F	F	CTACTGTTCAAGCCTCCAAG	Nested PCR, HBV L fragment
HB6R	R	AACAGACCAATTTATGCCTA	Nested PCR, HBV L fragment
1645F	R	AGGTCTTGCATAAGAGGACT	Nested PCR, HBV S fragment
2249R	F	CCAAAAGACACCAAATAYTC	Nested PCR, HBV S fragment
172A	F	ATCTCTAGCAGTGGCGCCCGAACAG	RT-PCR, HIV-1 *gag* fragment
173B	R	CTGATAATGCTGAAAACATGGGTAT	RT-PCR, HIV-1 *gag* fragment
174A	F	CTCTCGACGCAGGACTCGGCTTGCT	Nested PCR, HIV-1 *gag* fragment
175B	R	CCCATGCATTCAAAGTTCTAGGTGA	Nested PCR, HIV-1 *gag* fragment
K1	F	AAGGGCTGTTGGAAATGTGG	RT-PCR, HIV-1 *pol* fragment
U13	R	CCCACTCAGGAATCCAGGT	RT-PCR, HIV-1 *pol* fragment
K4	F	GAAAGGAAGGACACCAAATGA	nested PCR, HIV-1 *pol* fragment
U12	R	CTCATTCTTGCATATTTTCCTGTT	Nested PCR, HIV-1 *pol* fragment
106A	F	CATACATTATTGTGCCCCGGCTGG	RT-PCR, HIV-1 *env* fragment
17B	R	AGAAAAATTCCCCTCTACAATTAA	RT-PCR, HIV-1 *env* fragment
14A	F	AATGTCAGCTCAGTACAATGCACAC	Nested PCR, HIV-1 *env* fragment
10B	R	ATTTCTGGGTCCCCTCCTGAGG	Nested PCR, HIV-1 *env* fragment

[a] F, forward; R, reverse.

TABLE 2. HBV genotype reference sequences collected from the DNA Database of Japan (DDBJ) for tMRCA analysis

Genotype	DDBJ accession no.
A	FJ692588, GQ325786, GQ477503, GQ477496, GQ486599, EU414132
B	FJ751547, GQ924611
C	GQ924615, GQ486096, EU939589, GQ486684
D	GQ486337, FJ349228, GQ924652, EU414124, GQ922001, GQ486586
E	GQ486756, GQ161830, FJ349237
F	GQ486537, GQ486515, GQ486570
G	GQ486843
H	GQ486592, AB266536

followed by a second PCR using LA *Taq* polymerase. The primers used for HIV-1 sequencing are also summarized in Table 1. The amplicons were purified using a MultiScreen PCR filter plate (Millipore, Billerica, MA), and the sequencing reaction was performed using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Carlsbad, CA) and analyzed with the ABI PRISM 3130 (Applied Biosystems) autosequencer. Electropherograms were edited and verified by SeqScape v2.5 software (Applied Biosystems).

Phylogenetic tree analyses and genotype determination. HBV genotypes were determined by phylogenetic tree analysis with reference sequences. HBV sequences were aligned with 23 reference sequences from the National Center for Biotechnology Information (NCBI) database by using the CLUSTAL W program and analyzed by Kimura two-parameter methods. Genetic distances were calculated by the maximum composite likelihood, and phylogenetic trees were constructed by the neighbor-joining method using MEGA version 4 software. The reliabilities of branches were evaluated by bootstrap analysis with 1,000 replicates.

Phylogenetic trees of the HIV-1 *gag*, *pol*, and *env* regions were also constructed with 62 HIV-1 reference sequences obtained from the HIV-1 sequence database (Los Alamos National Laboratory).

Estimated tMRCAs. Evolutionary rates, chronological phylogenies, and other evolutionary parameters of HBV genotypes were estimated from heterochronous data for the HBV genomic sequences collected in our study, together with reference sequences from public databases (Table 2), using the Bayesian Markov chain Monte Carlo (MCMC) method. The nucleotide substitution model was evaluated by the hierarchical likelihood ratio test using PAUP v4.0 (29) with MrModeltest (14) and the general time-reversible (GTR) model with both invariant site (I) and gamma-distributed site (G) heterogeneity for four rate categories showing maximum likelihood. Bayesian MCMC analyses were performed with BEAST v1.4.8 (4) using the substitution model of GTR + I + G, three partitions into codon positions, and a relaxed molecular clock model (the uncorrelated log normal-distributed model) (3). Four different population dynamic models (exponential growth, logistic growth, constant population, and Bayesian skyline plot [BSP]) were tested in the analyses. According to BSP properties, constant-growth models were adopted for the HBV genome sequences. Each Bayesian MCMC analysis was run for 40 million states and sampled every 10,000 states. Posterior probabilities were calculated with a burn-in of 4 million states and checked for convergence using Tracer v1.4 (21). The maximum clade credibility tree for analyzing the MCMC data set was annotated by TreeAnnotator in the BEAST package. The posterior distribution of the substitution rate obtained from the heterochronous sequences was subsequently incorporated as a prior distribution for the mean evolutionary rate of the HBV genome, thereby adding a time scale to the phylogenetic histories of the given viruses and enabling estimation of the time of the most recent common ancestor (tMRCA) (19).

Determination of HBV drug resistance mutations. HBV cases resistant to nucleoside analogue reverse transcriptase inhibitors (NRTI) were determined by analyzing amino acid sequences of the RT region. The approved anti-HBV drugs in Japan are lamivudine, adefovir, and entecavir. In cases of HBV/HIV-1 coinfection, tenofovir and emtricitabine are also used. We studied whether the viruses have drug resistance mutations against these antiretroviral drugs with or without a history of antiretroviral treatments and confirmed the following resistance mutations: lamivudine/emtricitabine resistance mutations V173L, L180M, and M204I/V; adefovir resistance mutations A181V, I233V, and N236T; entecavir resistance mutations I169T, L180M, T184G, S202I, M204I/V, and M250V; and tenofovir resistance mutation A194T (1, 2, 24, 32, 34, 35). Furthermore, major drug resistance mutations in HIV-1 were defined according to the criteria of the International AIDS Society (IAS)-USA and Stanford HIV drug resistance database (7, 23).

RESULTS

The major HBV genotype circulating among Japanese MSM is genotype A. During the study period, 394 cases were newly diagnosed as HIV/AIDS, and 31 cases were determined as HBsAg positive. Thus, the average prevalence of HBV/HIV-1 coinfection in our study population was 7.9%. Analysis of the coinfection prevalence in each year showed increases from 2.8 to 3.3% in 2003 to 2004 and from 7.4 to 13.2% in 2005 to 2007 (Fig. 2). As the suspected route of HIV-1 infections in all 31 cases was MSM, HBV appears to be quickly spreading among the MSM population. Of these HBV/HIV-1-coinfected cases, 26 isolates were successfully sequenced for both HBV and HIV-1, and their subtypes and genotypes were determined. Regarding the five cases for which the HBV genome could not be sequenced, plasma HBV DNA copies were undetectable in four cases, and low ($10^{3.3}$ copies/ml) in one case.

The median age of the patients was 34 years (interquartile range [IQR], 29.5 to 37.0) (Table 3). The median plasma viral loads of HBV and HIV-1 were 4.4×10^8 (IQR, 4.9×10^4 – 6.3×10^8) and 6.4×10^4 (IQR, 2.0×10^4 – 2.0×10^5) copies/ml, respectively. Hepatitis B core antigen (HBcAg) IgM was positive in nine patients, of which two were suspected to harbor acute HBV infection according to their HBsAg positivity, AST and ALT plasma levels, and patient interviews. The other 7 HBcAg-positive patients were categorized as having acute hepatitis or exacerbated chronic hepatitis, and 17 HBcAg-negative patients were determined as being in the chronic hepatitis stage.

According to phylogenetic tree analysis, 26 cases were classified into two genotypes, either A or C. As shown in Fig. 3, 21 and 5 cases were classified as genotypes A and C, respectively. The subgenotypes of the 21 genotype A cases were all A2, the predominant subgenotype in Europe and North America, whereas the subgenotypes of the 5 genotype C cases were all C1, the most prevalent subgenotype in eastern Asia, including Japan, South Korea, and northern China. Genotype B, the

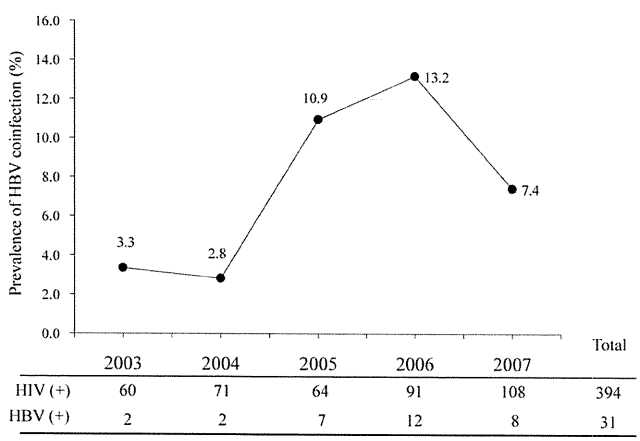

FIG. 2. Transitions in HBV infection rates in HBV/HIV-1-coinfected patients. HBV infection rates are plotted versus year, with the numbers of HIV-1-infected and HBV/HIV-1-coinfected patients shown below the *x* axis.

TABLE 3. Characteristics of HBV/HIV-1-coinfected patients

Characteristic	Value[a] for genotype:			P
	All (n = 26)	A (n = 21)	C (n = 5)	
Age (yr)	34 (30–37)	33 (29–37)	56 (46–57)	<0.01
Suspected route of HIV-1 infection	MSM	MSM	MSM	
AST (IU/liter)	31 (26–63)	29 (26–48)	54 (20–74)	
ALT (IU/liter)	43 (33–90)	42 (32–85)	44 (34–99)	
No. HBcAg IgM positive	9	9	0	<0.01
CD4 (/μl)	293 (91–492)	300 (94–484)	202 (9–494)	
HIV-1viral load (copies/ml)	6.4×10^4 (2.0×10^4–2.0×10^5)	6.8×10^4 (2.4×10^4–2.1×10^5)	2.4×10^4 (2.4×10^3–9.7×10^4)	
HBV viral load (copies/ml)	4.4×10^8 (4.9×10^4–6.3×10^8)	6.3×10^8 (4.7×10^4–6.3×10^8)	2.0×10^8 (4.7×10^5–6.3×10^8)	

[a] Median values are shown. Numbers in parentheses represent interquartile ranges.

second most predominant HBV genotype in Japan, was not detected in our study. Interestingly, the genotype A and C populations showed obvious differences in genetic diversity. The 21 group A2 samples (Fig. 3) formed a cluster with little

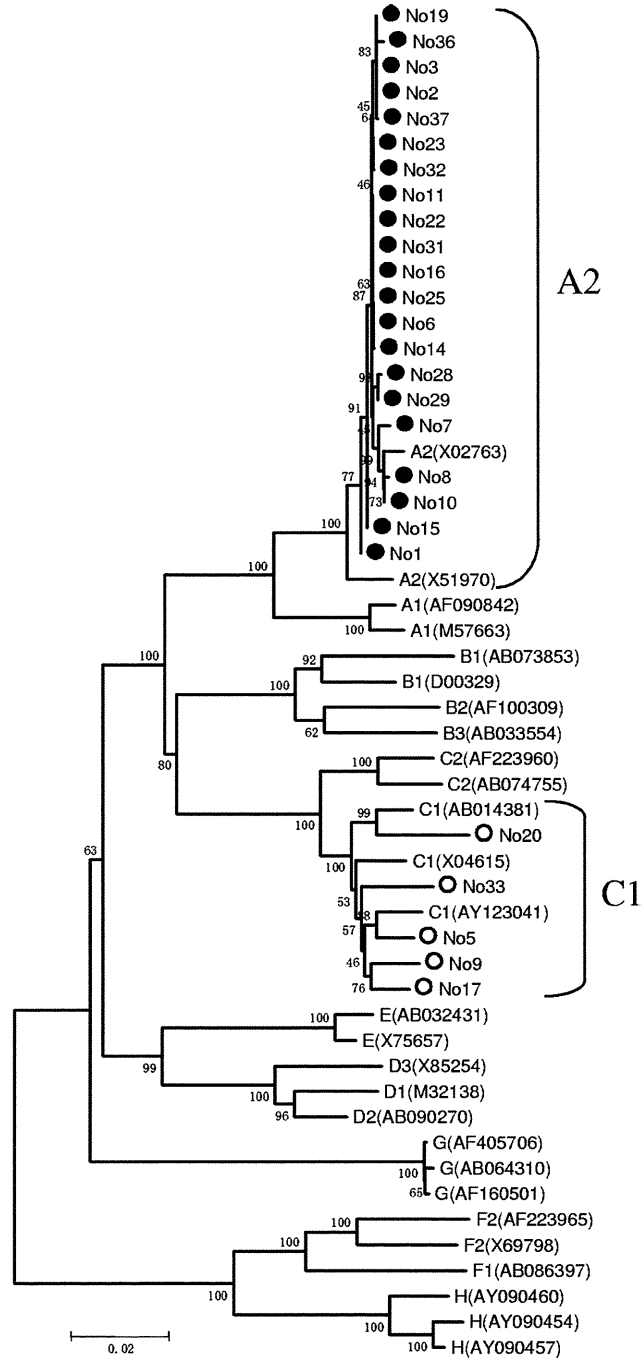

FIG. 3. Phylogenetic tree analyses of HBV isolated from HBV/HIV-1-coinfected patients. The phylogenetic tree was constructed using 26 full-length HBV genome sequences detected in HBV/HIV-1-coinfected patients in Nagoya (both solid and open circles) and 23 reference sequences from the NCBI database. Twenty-one and five cases were distributed in the clusters of genotype A (solid circles) and C (open circles), respectively.

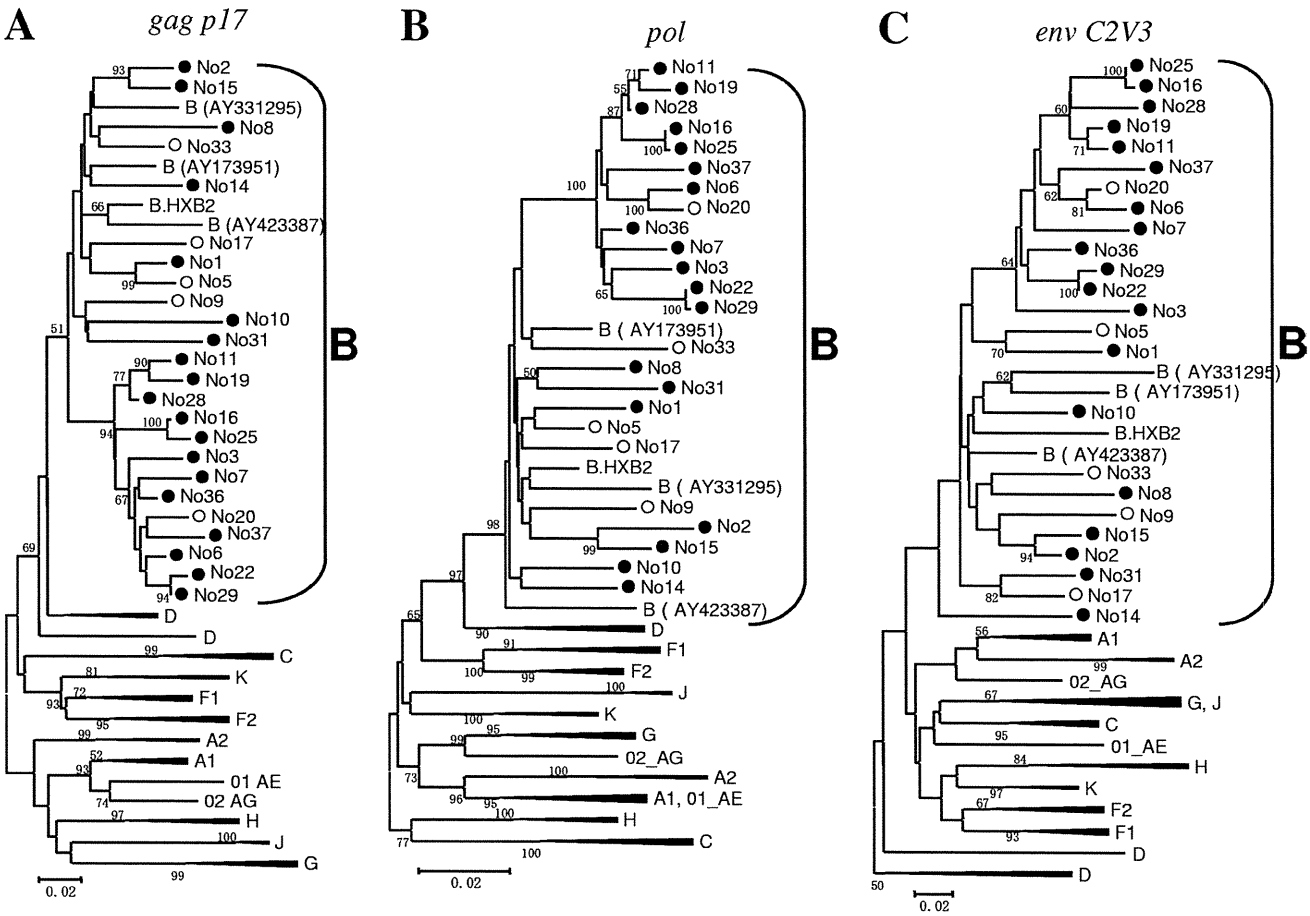

FIG. 4. Phylogenetic tree analyses of HIV-1 isolated from HBV/HIV-1-coinfected patients. Phylogenetic trees were constructed using the 25 HIV-1 sequences obtained in this study and 62 HIV-1 reference sequences from the Los Alamos National Laboratory database. The nucleotide base sequences of *gag p17* (A), *pol* PR to RT (B), and *env C2V3* (C) gene regions were analyzed. In all analyses, all the HIV-1-positive cases detected in Nagoya (both solid and open circles) were distributed in the subtype B cluster. Cases of coinfection with genotype C HBV are shown with open circles.

or no genetic distance between each other, indicating their extremely close genetic relationships. In contrast, the five group C1 cases did not form a single cluster and had longer branches than those of group A2.

Patients with genotypes A and C also differed significantly in age (Table 3). The median age of the genotype A patients was 33 years (IQR, 29 to 37), whereas that of the genotype C patients was 56 (IQR, 46 to 57) ($P < 0.01$). Furthermore, all nine HBcAg IgM-positive cases, including five suspected cases of acute infection, were categorized in genotype A2, suggesting ongoing active transmission of the virus among the Japanese MSM population. Thus, the genotype A2 population appeared to be younger, with more acute cases, and infected with an almost genetically identical HBV strain. These two genotypes did not differ significantly in regard other clinical data, such as AST and ALT levels, $CD4^+$ T cell count, and HBV and HIV-1 viral loads.

To clarify the detailed epidemiological features of HBV/HIV-1-coinfected patients, the HIV-1 subtypes and their genetic distances were determined by phylogenetic analyses of three genome regions, *gag p17*, *pol*, and *env C2V3*. All 26 samples were determined as subtype B (Fig. 4A, B, and C), and interestingly, branch patterns and relationships among cases were different from those for HBV. There were six paired cases, demonstrating a significantly close genetic relationship (>50% bootstrap value) in more than two regions. These paired cases were cases 1 and 5, 2 and 15, 6 and 20, 11 and 19, 16 and 25, and 22 and 29, and these connections were not evident in the HBV phylogeny, suggesting different origins of sexual partner between the two pathogens in each pair. An alternative explanation could be that little genetic variation in HBV made it difficult to clarify the genetic relationships between cases. However, there was one discordant pair (cases 1and 5); one case had HBV genotype A, and the other case had HBV genotype C2. Furthermore, the other four HBV genotype C2 cases (cases 9, 17, 20, and 33) were scattered among HIV-1 phylogenies within genotype B HIV-1-infected patients.

HBV strains detected in HIV-1-infected patients from Nagoya are the same viruses found in other parts of Japan. To clarify whether the dominance of genotype A HBV in HIV-1-infected MSM is a regional issue in the Nagoya urban area or a more nationwide epidemic, we reconstructed an HBV phylogenetic tree of 26 cases together with HBV sequences col-

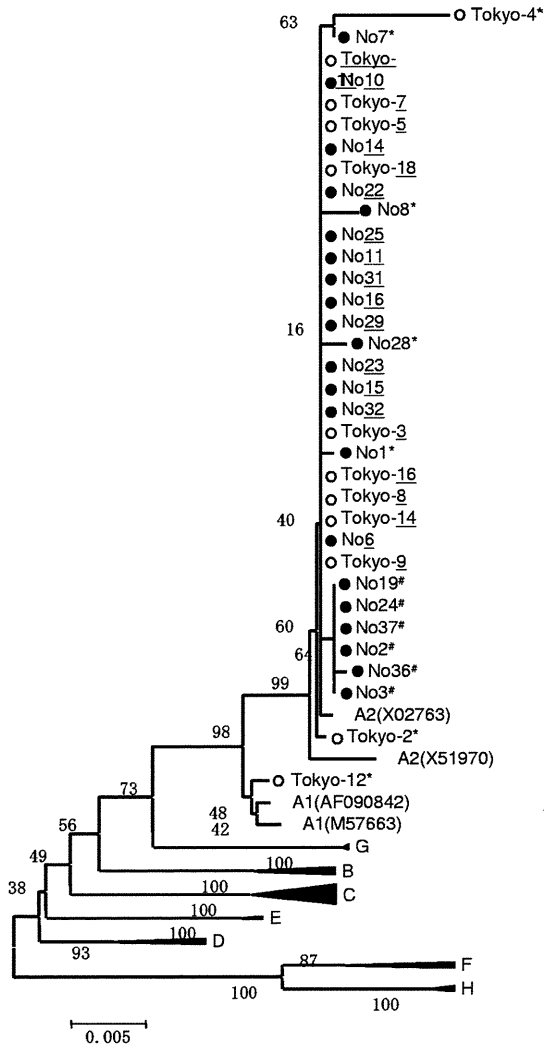

FIG. 5. Phylogenetic tree analysis of 35 HBV region S sequences, 22 from Nagoya (solid circles) and 13 from Tokyo (open circles). Three genetically different groups are indicated by asterisks, pound signs, and underlining.

lected at a different time and from a different area of Japan, i.e., 12 genotype A sequences from HBV/HIV-1-coinfected patients collected in Tokyo about 10 years before this study (8). As no full genome sequences were available for the Tokyo cases, only the S gene (681 bp [bp 155 to 835]) was analyzed. From the phylogenetic tree pattern, genotype A was classified into three groups (Fig. 5). The first is a group of 21 identical sequences (underlined in Fig. 5). As this group had the largest number of cases and included sequences from both Nagoya and Tokyo, this strain appears to be prevailing nationwide. The second group is a cluster of cases, i.e., cases 2, 3, 19, 24, 36, and 37. As all six cases were from Nagoya, this isolate still seems to be in an endemic status. The third group comprises isolates with longer branches (noted by asterisks), i.e., Tokyo-2, -4, and -12 and Nagoya-1, -7, -8, and -28. These isolates appear to be quite distinct from the others, suggesting that their origin may not be sexual contact but another route, such as MTCT or transfusions.

The prevailing HBV genotype A2 emerged more recently than most other genotypes. To estimate the emergence time of the prevailing genotype A2 strain, we estimated its mutation rate per year and tMRCA. First, the median mutation rate per year was calculated as 3.23×10^{-5} (5.62×10^{-8} to 9.01×10^{-5}), which is close to those previously reported (10, 18). Next, the median tMRCAs of all A strains, A1, A2, and C were determined to be 370.8, 88.9, 184.3, and 494.9 years ago, respectively (Table 4; Fig. 6). Thus, the A2 genotype is one of the youngest HBV genotypes.

A lamivudine resistance amino acid HBV mutation detected in an antiretroviral therapy-naïve patient. We clarified not only HBV genotypes but also the incidence of transmitted drug-resistant HBV among the study patients. Analysis of the amino acid sequence of the HBV RT region showed a combined triple amino acid mutation, rtV173L + rtL180M + rtM204V, which was a mutation causing resistance against lamivudine and its 5-fluoro analogue (2′,3′-dideoxy-3′-thia-5-fluorocytidine), in two patients (patients 5 and 8). However, one patient (patient 5) had been treated with stavudine-lamivudine-efavirenz at the time of sample collection, and thus only one case (case 8) was suspected to be a transmitted HBV drug-resistant case. No HIV-1 drug-resistant virus transmission was detected in the study sample.

DISCUSSION

This molecular epidemiological study of HBV infection in HIV-1-seropositive patients revealed epidemiological characteristics that were unique compared to those of the general population in Japan. All HBV/HIV-1-coinfected patients were MSM, they had a 10-fold-higher prevalence (7.9%) than that of the general population, and genotype A was the predominant HBV genotype (31). This distinct HBV epidemic in MSM was first reported in 2001 in other regions of the country (9, 36), a decade before our study. Furthermore, phylogenetic analysis of sequences from the two studies, collected in different regions and years, revealed that an identical genotype A strain prevails among the MSM population nationwide.

Considering the status of HBV epidemiology in the general population of Japan, genotypes C and B must have an equal or greater chance to disseminate among the HIV-1-seropositive

TABLE 4. Estimated times of the most recent ancestor (tMRCAs) for HBV genotypes

Genotype	Mean tMRCA (yr before)		95% HPD[a]	
	Mean	Median	L	H
A	1,294.2	370.8	27.1	4,046.4
A1	306.6	88.9	12.4	976.4
A2	597.4	184.3	18.8	1,886.2
B	345.8	88.5	4.2	1,069.3
C	1,655.3	494.9	36.6	5,124.7
C2	1,062.4	308.6	20.8	3,296.6
D	827.2	226.6	11.2	2,469.4
E	163.7	38.9	4.5	539.7
F	1,060.8	308.2	13.7	3,277.4
H	433.8	110.1	5.6	1,303.0

[a] HPD, highest posterior density.

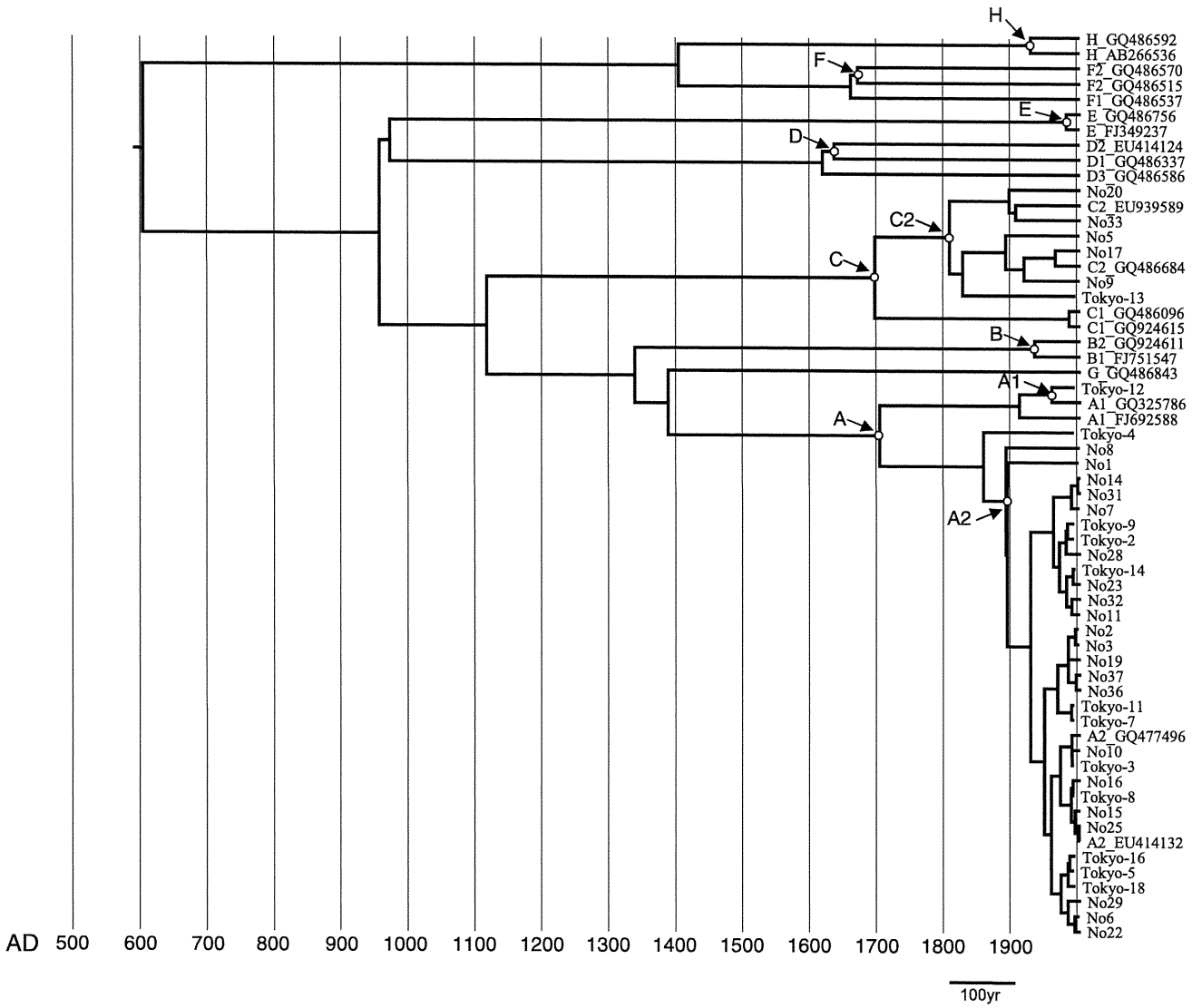

FIG. 6. Maximum clade credibility tree depicted according to median tMRCA. Nodes with open circles are evaluated points for each genotype summarized in Table 2.

MSM population. In fact, we found five genotype C patients in our study sample, and all five patients were MSM. However, because these five genotype C patients were older and their isolates had longer branches in phylogenetic analysis than the prevailing genotype A isolates, they appear to have been independently infected through either MTCT or blood transfusion events rather than sexual contact. Furthermore, as all five cases were singletons without any genetically close isolates among the samples analyzed, this genotype appeared to be less efficiently transmitted by sexual contact.

Interestingly, predominant genotype A HBV coinfection in HIV-seropositive MSM populations has also been reported in European and South American countries (20, 26, 33), suggesting that the prevailing genotype A in HIV-seropositive MSM has become a worldwide HBV epidemic. Regarding HBV genotypes in the HIV-negative population in Japan, genotype A has been increasing, but the major HBV genotype is still C, with genotype A remaining at 3.5% nationwide and 2.1% in the Tokai area, which includes Nagoya city (9). Therefore, the prevalence of genotype A HBV in the MSM population is significantly higher than in the rest of the population.

Thus, it is interesting to discuss the virological advantages disposing this genotype A isolate to become the major player in HBV/HIV-1 coinfection among MSM. One such advantage might be the higher progression rate (16 to 23%) to chronicity of genotype A than of genotype C (28, 30), enhancing its capacity to serve as a source of new infections. As 9 of 26 genotype A-infected patients (35%) were HBcAg IgM positive and 2 had acute hepatitis, it is obvious that genotype A infections are actively ongoing among the MSM population. Though further studies are needed, considering the tMRCA of the prevailing strain A2, the younger age of patients infected with this strain than of those infected with other genotypes,

and its high prevalence among MSM, this strain may have acquired higher infectivity and efficient transmission through sexual contact.

Another issue we wanted to clarify in this study was the transmission of antiviral drug resistance. We found no antiretroviral resistance in the 26 sequenced cases. On the other hand, we detected two cases with a mutation combination of rtV173L + rtL180M + rtM204V in HBV reverse transcriptase, demonstrating resistance against lamivudine-emtricitabine. One patient was antiretroviral therapy naïve; thus, transmission of drug-resistant HBV is strongly suspected. It is peculiar that the isolate harboring the drug-resistant mutations in HBV was a singleton, considering that genetically identical isolates were prevailing, that there were very low mutation rates that suggest few chances of reverting to wild type, and that there were actively ongoing *de novo* infections. This finding might be due to resistant viruses being masked by wild-type viruses under untreated conditions, as reported in the case of HIV-1 drug resistance (6). The possibility of minority resistance populations of HBV could be verified by detection with a highly sensitive method.

In conclusion, we clarified the molecular epidemiology of HBV/HIV-1 coinfection in Japan. Our data suggest that ongoing HBV infections lie outside prevention programs targeting the MTCT and blood transfusion infection routes, and they suggest the urgent need for new prevention strategies focusing on the high-risk group of the HIV-1-seropositive MSM population.

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ORIGINAL ARTICLE

Investigation of emtricitabine-associated skin pigmentation and safety in HIV-1-infected Japanese patients

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Abstract Emtricitabine (FTC) has been reported to cause skin pigmentation (SP), and the incidence of SP associated with FTC varied with ethnicity, with a higher rate in African-American patients (8%). We assessed the incidence of SP in Japanese HIV-1-infected patients receiving combination antiretroviral therapy (cART) with FTC for a period of 48 weeks and confirmed new findings of FTC-associated SP, including pathological characteristics. This was a multicenter, prospective, longitudinal non-randomized study. We evaluated the appearance of SP at 48 weeks as the primary endpoint in 155 Japanese patients, and secondary endpoints included the characteristics of the SP (location, color tone, size, and progression). Six cases (3.9%) of SP occurred at a median of 124 days (range: 7–259 days) within 48 weeks. The SP looked like an isolated dark spot, 1–2 mm in diameter, mainly on the hands and/or feet. The severity of all the SPs was mild. Each SP had disappeared or faded at a median of 112 days (range: 28–315 days) with continued FTC. FTC-associated SP was considered to be lentigo simplex by dermatoscopy and pathological appearance. In summary, the incidence of FTC-associated SP in Japanese patients was 3.9%, and was comparable to the previously reported incidence in Asian patients (4%). FTC-associated SP was not associated with any clinically significant symptoms and has little clinical significance.

Keywords Emtricitabine · Skin pigmentation · Japanese · HIV-1

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Introduction

Emtricitabine (FTC) is a cytidine analogue nucleoside reverse-transcriptase inhibitor (NRTI) that is listed in various guidelines as a recommended component of the initial antiretroviral treatment regimens for HIV-infected patients [1, 2]. FTC is a preferred choice in Japan, and is often administered concomitantly with tenofovir disoproxil fumarate (TDF). In the treatment of HIV infection, the use of once-daily medications has increased in recent years, and the total number of medications taken each day has decreased; this has helped to maintain compliance with drug regimens and improve the performance of combination antiretoroviral therapy (cART). However, there are various adverse events associated with cART, and controlling these adverse events is important, as they can make

it more difficult to maintain compliance and may lead to medication changes or the discontinuation of cART.

FTC was reported to cause skin pigmentation in phase 3 clinical trials, and the incidence of skin pigmentation was higher in individuals of non-Caucasian ethnicity (African-American: 8%; Asian: 4%; Hispanic: 3%; Caucasian: less than 1%) [3]. Most of this skin pigmentation occurred on the palms of the hands or soles of the feet, and the severity was mild to moderate in all cases; FTC use was thus continued [3]. However, there are no reports detailing the clinical features and outcomes of patients with this symptom, such as the causes of the FTC-associated skin pigmentation and possible influence on recovery or cART continuation. We therefore examined Japanese patients infected with HIV-1 and confirmed new findings of FTC-associated skin pigmentation, including its incidence and pathological characteristics. In addition, we investigated the effectiveness of FTC, and whether it has adverse events specific to Japanese patients. Furthermore, TDF was also used as an another NRTI component in a dual NRTI component of cART by all subjects in the present study. Although the effectiveness and safety of TDF have been demonstrated previously, the number of reports of renal tubular dysfunction associated with TDF has increased, leading to concerns about risks of renal toxicity in long-term TDF use. We therefore examined the renal function of antiretroviral-naïve Japanese patients, whom we were able to observe for 96 weeks of TDF therapy.

Patients and methods

This prospective, open-label trial commenced at 6 institutions in June 2005. The subjects were Japanese HIV-1-infected patients with no history of FTC use; previous treatment with other medications was disregarded. FTC (Emtriva® capsules 200 mg or Truvada® tablets, a fixed-dose of FTC and tenofovir, Gilead Sciences, Foster city, CA, USA) was used concomitantly with a nonnucleoside reverse-transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI) as the key third agent. Pictures of the palms of both hands were taken prior to FTC treatment. Both palms were visually inspected for onset of skin pigmentation during each patient's hospital visits (about every 1–2 months). Skin pigmentation onset in areas other than the palms was investigated to the fullest possible extent by patient interview or visual inspection. We observed subjects for skin pigmentation onset for 48 weeks or longer. The primary endpoint of this study was the incidence of skin pigmentation at 48 weeks; secondary endpoints included the characteristics of the skin pigmentation (location, color tone, size, and progression), the incidence of adverse events, and virological/immunological effects. If skin pigmentation occurred, subjects were observed either until the symptoms resolved or until 1 year had passed following their occurrence. The time until skin pigmentation onset, resolution, or fading was calculated, whether recognized by the patient or diagnosed by a doctor. Detailed examinations were performed by a dermatologist as needed, and included observation by dermoscopy or histological examination by biopsy in two cases.

As a post-hoc analysis, we examined renal function in antiretroviral-naïve patients who had their creatinine levels measured at 96 weeks and virological/immunological effects in all naïve patients. Renal function was evaluated by calculating the estimated glomerular filtration rate (eGFR), using the Modification of Diet in Renal Disease (MDRD) equation. To investigate changes in eGFR over 96 weeks, we performed a one-way analysis of variance with time as the factor, as well as a two-way analysis of variance with time and medication as factors. Statistical significance was set at $p < 0.05$.

MDRD equation

$$\mathrm{GFR(mL/min/1.73\ m^2)} = 186 \times [\text{serum creatinine (mg/dL)}]^{-1.154} \times [\text{age(years)}]^{-0.203} \times [0.742 \text{ if female}] \times [1.212 \text{ if black}]$$

Results

Study population

This study included a total of 155 patients, 72 of whom were undergoing initial treatment for HIV-1 infection (naïve patients), and 83 of whom had undergone previous treatments (experienced patients). All experienced patients were changed to an FTC + TDF component from different NRTI components. One experienced patient was treated with FTC + TDF (single-ingredient drugs) and all other patients received a fixed-dose formulation of Truvada. The baseline characteristics of the naïve and experienced patients are shown in Table 1. At the beginning of this study, 37.5% of the naïve patients had a viral load of HIV RNA of ≥100,000 copies/mL. At the beginning of this study, 14 experienced patients had a viral load of ≥400 copies/mL, and the other 69 experienced patients had HIV RNA levels of <400 copies/mL. Medicines used as a key third agent were similar in both the naïve and experienced patients, with approximately 40% using an NNRTI and about 60% using a ritonavir-boosted PI (Table 1).

Ten patients withdrew from the study within 48 weeks of the start. Of these, 3 patients withdrew due to adverse events, 2 withdrew due to their doctor's decisions (1 patient:

Table 1 Baseline characteristics of the patients

Variable	Naïve patients ($n = 72$)	Experienced patients ($n = 83$)
Age (years)[a]	35 (20–66)	39 (24–67)
Male	71 (98.6%)	77 (92.8%)
HIV RNA (copies/mL)[a]	65,000 (1,100–3,600,000)	<50 (<50–1,100,000)
CD4 count (cells/mm^3)[a]	174 (1–289)	419 (37–1,163)
Key third agent		
EFV	27 (38.5%)	35 (42.2%)
ATV + rtv	33 (45.8%)	36 (43.4%)
LPV/rtv	10 (13.9%)	11 (13.3%)
FPV + rtv	2 (2.8%)	1 (1.2%)

EFV efavirenz, *ATV* atazanavir, *LPV* lopinavir, *FPV* fosamprenavir, *rtv* low-dose ritonavir

[a] Median (range)

Table 2 Summary of skin pigmentation findings in FTC-treated Japanese patients

Case no.	Age (at the beginning, years)	Sex	Key third agent	Time to onset (days)	Number of SPs	Location	Outcome
1	26	Male	EFV	119, 175, 203, 259, 343	9	Dorsum of right hand, precordium, dorsum of foot, forearm	Fading and resolution (dorsum of hand: base of thumb)
2	33	Male	EFV	154, 175	4	Precordium, shoulder, dorsum of hand	Fading (dorsum of hand)
3	32	Male	ATV + rtv	73, 94, 129, 220, 402	11	Palm and dorsum of right hand, sole of right foot, face[a]	Fading and resolution (location not identified)
4	34	Male	ATV + rtv	21	1	Palm of right hand	Resolution
5	45	Male	EFV	91	1	Palm of left hand	Resolution
6	23	Male	LPV/rtv	7, 94, 150	>50	Palms of both hands, dorsum of right hand	Fading (palm of right hand)

FTC emtricitabine, *SP* skin pigmentation, *EFV* efavirenz, *ATV* atazanavir, *LPV* lopinavir, *rtv* low-dose ritonavir

[a] Because fleck and lentigo may have developed due to other causes, it was difficult to define whether SPs on the face were due to FTC

medication change because of vomiting and nausea, not adverse events; 1 patient: withdrew due to exacerbation of anal canal cancer), 1 withdrew due to virological failure, and 4 were lost to follow-up.

Primary endpoint

Six of the 155 patients reported skin pigmentation, resulting in an incidence of 3.9% at 48 weeks.

Secondary skin endpoints

The median time until skin pigmentation onset within 48 weeks was 124 days (range: 7–259 days). At more than 48 weeks, there were two cases where skin pigmentations occurred (at 343 days and 402 days, respectively). All the cases of skin pigmentation were grade 1 on the Division of AIDS severity grading scale (mild, slight, or localized) and did not require treatment. The median number of skin pigmentations was 6.5 (range: 1 to >50; Table 2). The size of individual pigmentations did not appear to increase with time; the majority were 1–2 mm in diameter and circular, with the color ranging from brown to black. Pigmentation occurred in multiple areas, particularly on the palm and dorsum of the hand (Table 2). FTC treatment was continued in all patients with pigmentation, and the pigmentation resolved in 4 patients and faded in 3 (one patient showed both resolution and fading of pigmentation). Median time before the resolution or fading of pigmentation was 112 days (range: 28–315). No patients discontinued FTC treatment because of skin pigmentation.

Figure 1 shows a typical example of skin pigmentation that occurred on the palms within 3 weeks of the start of FTC use (Fig. 1b), and resolved after 7 weeks (Fig. 1c). The characteristics of the skin pigmentations that occurred on the dorsum of the hand were identical to those on the palm, and some pigmentations resolved while the patient was continuing FTC use (Fig. 1d, e).

In one patient, we examined the pigmentation on the palm of the hand by dermoscopy; the pigment network was uniform, with increased melanin levels in the epidermis (Fig. 2). We performed a biopsy of a pigmented area located on the dorsum of the hand in another patient. Pathological findings indicated increased melanin levels in the basal layer of the epidermis, similar to lentigo simplex.

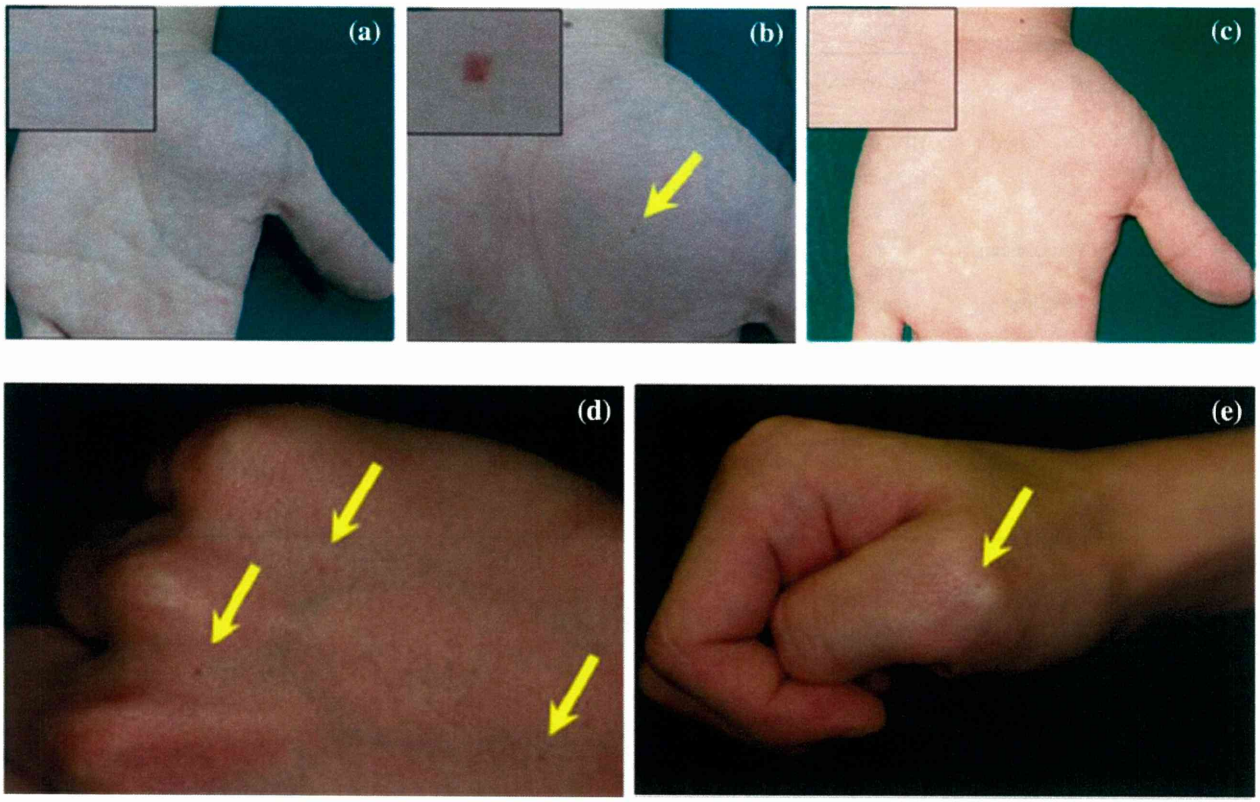

Fig. 1 Skin pigmentation (typical cases). Palm of hand; Case no. 4. **a** Prior to emtricitabine (FTC) therapy, **b** skin pigmentation (*arrow*) occurred after 3 weeks of FTC therapy, **c** skin pigmentation had resolved after 7 weeks of FTC therapy. Dorsum of hand; Case no. 1. **d**, **e** Skin pigmentation (*arrows*) on dorsum of hand (base of thumb) occurred after 25 weeks of FTC therapy and had resolved after 45 weeks of FTC therapy

Fig. 2 Observation, by dermoscopy, of skin pigmentation on the palm of the right hand. Case no. 6. The pigment network was uniform. Melanin was increased in the epidermis. The pathological finding of this skin pigmentation (on the palm of the hand) was expected to be similar to findings of skin pigmentation on the dorsum of the hand

Secondary safety endpoints

The subjects examined for the safety assessment were patients who had received at least one FTC treatment and had at least one safety evaluation.

Table 3 Summary of adverse events occurring within 48 weeks

Adverse events	Number
Any adverse event—no.	15
Skin pigmentation	6
Increased blood creatinine	2
A sense of abdominal fullness	1
Flatulence	1
Rash	1
Acute urticaria	1
Disseminated erythematous papules	1
Drug rash	1
Taste disturbance	1

Adverse events were seen in 15 of the 155 patients within 48 weeks of starting treatment (Table 3). As previously mentioned, 6 of these patients were of skin pigmentation. Other adverse events included: increased blood creatinine ($n = 2$); a sense of abdominal fullness (1); flatulence (1); rash (1); acute urticaria (1); disseminated erythematous papules (1); drug rash (1); and taste disturbance (1). Study drug administration was

discontinued in 3 of these patients due to adverse events (increased blood creatinine, a sense of abdominal fullness, and rash).

Secondary efficacy endpoints

Virological effects were examined in patients who had received at least one FTC treatment and had their viral load measured at least once. Data were analyzed by an intent-to-treat analysis (missing = failure).

Forty-eight weeks after starting treatment, 86% (60/70) of the naïve patients had a viral load of <400 copies/mL. Of the experienced patients who had viral loads of ≥400 copies/mL at the beginning of the study, 71% (10/14) had <400 copies/mL after 48 weeks. Of the experienced patients who had a viral load of <400 copies/mL at the beginning of the study, 87% (60/69) maintained a viral load of <400 copies/mL after 48 weeks.

Immunological effects were examined in patients receiving FTC treatment for 48 weeks or longer and who had their CD4 cell count measured before and after 48 weeks of treatment. In naïve patients ($n = 62$), the mean change seen in CD4 cell counts after 48 weeks was 193 ± 12.8 (mean ± SE) cells/mm^3. In experienced patients who had initial viral loads of ≥400 copies/mL of HIV RNA ($n = 9$), the mean change in CD4 cell counts after 48 weeks was 168 ± 49.4 (mean ± SE) cells/mm^3. In experienced patients who had initial viral loads of <400 copies/mL of HIV RNA ($n = 58$), the mean change in CD4 cell counts after 48 weeks was 51 ± 17.0 (mean ± SE) cells/mm^3.

Post-hoc analysis

We evaluated renal function in 54 naïve patients who had their creatinine levels measured at 96 weeks.

In these 54 patients, the median serum creatinine level was 0.715 (range: 0.45–1.6) mg/dL at the beginning of treatment, and 0.785 (0.44–1.19) mg/dL after 96 weeks. The median eGFR in these 54 patients was 129 (range: 55–225) mL/min/1.73 m^2 at the beginning of treatment, 120 (76–215) mL/min/1.73 m^2 at 24 weeks after the beginning of treatment, 116 (77–219) mL/min/1.73 m^2 at 48 weeks, 120 (74–265) mL/min/1.73 m^2 at 72 weeks, and 123 (76–224) mL/min/1.73 m^2 at 96 weeks. We performed a one-way analysis of variance using time as the factor; and the results showed a significant difference between the start of treatment and 24 weeks after the start of treatment ($p < 0.05$), but the differences were not significant over other time periods. Patients who were concomitantly using NNRTIs ($n = 17$) had an eGFR of 139 (90–225) mL/min/1.73 m^2 at the beginning of treatment, 137 (76–215) mL/min/1.73 m^2 at 24 weeks after the beginning of treatment, 131 (85–197) mL/min/1.73 m^2 at 48 weeks, 124 (80–177) mL/min/1.73 m^2 at 72 weeks, and 132 (84–185) mL/min/1.73 m^2 at 96 weeks; there were no significant differences detected between the time periods. Patients who were concomitantly using PIs ($n = 37$) had an eGFR of 127 (55–204) mL/min/1.73 m^2 at the beginning of treatment, 115 (79–157) mL/min/1.73 m^2 at 24 weeks after the beginning of treatment, 114 (77–219) mL/min/1.73 m^2 at 48 weeks, 120 (74–265) mL/min/1.73 m^2 at 72 weeks, and 117 (76–224) mL/min/1.73 m^2 at 96 weeks; there were no significant differences detected between the time periods. We performed a two-way analysis of variance using time and medication as factors in order to compare patients concomitantly using NNRTIs and those concomitantly using PIs. No significant differences were seen in the interaction between time and medication.

Of the naïve patients, 76% (53/70) had a viral load of <400 copies/mL after 96 weeks. The mean change in CD4 cell count was 222 ± 18.0 (mean ± SE) cells/mm^3 ($n = 55$).

Discussion

In the present study, the incidence of FTC-associated skin pigmentation in Japanese patients was 3.9%, which is comparable to the previously reported incidence in Asian patients (4%). Skin pigmentation was primarily seen on the palms and dorsum of the hands. With the exception of one patient, 1–11 pigmented areas were seen in each instance of pigmentation; there was no tendency for the patches to grow in size over time. Symptoms of FTC-associated skin pigmentation were generally mild. In all cases of skin pigmentation, symptoms resolved or faded while the patients were continuing FTC use, thus suggesting that this condition is reversible.

Previous reports on antiretroviral drugs have indicated that diffuse and progressive nail hyperpigmentation may occur due to zidovudine (ZDV) use [4, 5]. Affected areas include the nails, the palms of the hands, lips, and oral mucosa. Previous reports indicate that ZDV-associated hyperpigmentation progresses from the proximal nails to distal nails over time. Depending on the case, pigmentation may then spread to the tongue, the nail of the fourth toe, and the abdomen. In addition, results suggest that the progression of ZDV-associated hyperpigmentation differs with ethnicity. The degree and extent of nail pigmentation correlate with ethnicity; that is, Caucasians are unaffected, Hispanics develop blue lunula with longitudinal bands, and African-Americans show diffuse, brown-black nail hyperpigmentation. It has also been reported that this hyperpigmentation is reversible by reducing or discontinuing ZDV use [6].

Previously, FTC-associated skin pigmentation was reported as palmar freckling in African-American patients [7]. Previous reports have also indicated that there is a higher incidence of pigmentation in non-Caucasian patients; however, these reports did not indicate that the degree of pigmentation was dependent on ethnicity. In the present study, the skin pigmentations observed in Japanese patients were primarily 1–2 mm in diameter, with color ranging from brown to black. Symptoms did not intensify during the observation period and there have been no reports of cases requiring additional treatment. Of the 6 patients of skin pigmentation in this study, 4 were in naïve patients; the other 2 patients had been changed from other NRTI treatments. One of these experienced patients was changed from a treatment containing ZDV and lamivudine; however, we were unable to find any indication of nail hyperpigmentation (which is commonly seen in patients taking ZDV) in this patient, and the influence of prior treatment with ZDV remains unclear.

When areas of ZDV-associated hyperpigmentation were examined using biopsy, they showed increased melanin in the basal layers and within melanophages in the dermis. We examined FTC-associated pigmentations by dermoscopy and biopsy; the results of both methods indicated increased levels of melanin similar to lentigo simplex. These findings indicate that FTC-associated pigmentation is similar to ZDV-associated hyperpigmentation.

Although we tried to detect risk factors of FTC-associated skin pigmentation, we were not able to clarify any such factors in the present study.

No clinically significant symptoms of FTC-associated skin pigmentation were observed in this study; FTC treatment was continued and it appears that this phenomenon is not clinically significant. We also observed that scarring from syphilitic lesions on the palm resembled FTC-associated skin pigmentation in one patient. In addition, it is possible that the skin pigmentations on other areas such as the dorsum of the hands, as well as the arms and face, could be everyday melasmas or lentigos. It should be noted that careful differentiation between these possible manifestations is required.

In regard to the secondary endpoints and post-hoc analysis in our study, the NRTI backbone showed favorable outcomes through cART containing FTC and TDF.

Adverse events were observed in 15 patients during the safety evaluation. Adverse events other than skin pigmentation were mostly dermatological symptoms such as rashes (4 of 15 cases). Other symptoms related to TDF use were increased blood creatinine, a sense of abdominal fullness, and flatulence. The adverse events observed in this study have all been previously recognized as expected adverse events of cART containing FTC and TDF; no adverse events appeared to be specific to Japanese patients.

When we compared the concomitant uses of NNRTIs and boosted-PIs, we saw no significant differences in eGFR changes over time due to concomitant drug use up to 96 weeks after the start of treatment. Interactions between certain PIs and TDF, which appear to result in reduced renal function, have also been reported [8–10]. However, in the present study, we found no evidence that the concomitant use of boosted PIs exacerbated the deterioration of renal function. Under both conditions, i.e., the concomitant uses of NNRTIs and boosted-PIs, the changes in median eGFR were within the normal ranges and there was no clinical evidence of significant deterioration.

With regard to the virological effect, 86% of the naïve patients had viral loads below the detection limit (<400 copies/mL) at 48 weeks; and even at 96 weeks, 76% were below the detection limit. In addition, 71% of the experienced patients who were changed to this treatment as salvage therapy and 87% of the patients whose viral load was under control at the beginning of treatment had a viral load below the detection limit at 48 weeks. Immunological testing indicated an increased CD4 cell count in both naïve and experienced patients (mean change: 50–200 cells/mm^3). These findings are similar to the results of previous reports [11–13], and this study provides evidence that cART containing FTC and TDF is effective in controlling symptoms both virologically and immunologically in Japanese patients infected with HIV-1.

In summary, the incidence of FTC-associated skin pigmentation in Japanese patients was 3.9%. The skin pigmentation that appeared during FTC treatment was not associated with any clinically significant symptoms. As FTC use was continued, it is thought that this phenomenon has little clinical significance. The high level of effectiveness and lack of severe adverse events specific to Japanese patients suggest that cART containing FTC is an effective HIV treatment option for Japanese patients.

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Conflict of interest All authors: no conflicts.

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CASE REPORT

Immune reconstitution to parvovirus B19 and resolution of anemia in a patient treated with highly active antiretroviral therapy

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Abstract Immune reconstitution inflammatory syndrome (IRIS) is an unsolved problem in the treatment of human immunodeficiency virus (HIV)-1 infection. Despite the high seroprevalence of parvovirus B19 (PVB19) among HIV-1-positive patients, reports on PVB19-induced anemia, especially that associated with PVB19-related IRIS, in these patients are limited. We present the case of a man with acquired immunodeficiency syndrome who developed severe transfusion-dependent anemia and was seropositive and borderline positive for immunoglobulin-M and IgG antibodies against PVB19, respectively. PVB19-DNA was also detected in his serum. The patient was diagnosed with pure red cell anemia (PRCA) caused by a primary PVB19 infection and was treated with periodical blood transfusions. However, he subsequently tested negative for IgG antibodies and developed chronic severe anemia with high levels of PVB19 viremia. This indicated a transition from primary to persistent infection. After initiation of highly active antiretroviral therapy, the patient showed an inflammatory reaction with rapid deterioration of anemia and seroconversion of the IgG antibody to PVB19. Subsequently, PRCA was completely resolved, but the patient's serum still contained low levels of PVB19-DNA. Thus, this was a case of IRIS associated with PVB19 infection. Our report highlights the significance of seroconversion to PVB19 in the diagnosis of IRIS and re-emphasizes the finding that persistently high levels of PVB19 viremia after primary infection are probably because of the lack of protective antibodies.

Keywords HIV-1 infection · Parvovirus B19 · Pure red cell anemia · Immune reconstitution inflammatory syndrome

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Introduction

Human immunodeficiency virus (HIV)-1 is known to infect $CD4^+$ T lymphocytes and cause acquired immunodeficiency syndrome (AIDS) by decreasing the number of $CD4^+$ cells. In the mid-1990s, a new and specific treatment, namely, highly active anti-retroviral therapy (HAART), was developed to treat HIV-1 infection; HAART is a combination therapy comprising administration of two or three classes of antiretroviral drugs. This therapy induces long-term suppression of viral proliferation and immunological reconstitution in HIV-1-infected patients and thus increases their survival rate. Although HAART cures opportunistic infections by restoring the immune system, it can also induce an inflammatory reaction that is characterized by the aggravation of a preexisting opportunistic infection and the emergence of other infectious diseases that were not observed before the initiation of HAART. This phenomenon, termed as immune reconstitution inflammatory syndrome (IRIS), is thought to be caused by an immunological reaction to a pathogen that was present in the host before the antiviral therapy [1]. This paradoxical syndrome poses a major problem in the patients who undergo HAART.

Human parvovirus B19 (PVB19) belongs to the genus *Erythrovirus*. PVB19 is the predominant pathogenic erythrovirus in humans and is the prototype strain for

genotype 1 [2]. PVB19 has been shown to cause erythema infectiosum in children as well as acute red cell aplasia in patients who have conditions causing hematopoietic stress, such as hemolytic anemia; this virus has also been implicated in the pathogenesis of rheumatic arthritis, myocarditis, nephritis, fulminant liver disease, and many other conditions [3]. In HIV-1-positive patients, PVB19 may persistently infect erythroid precursor cells, evade elimination by the immune system, and cause transfusion-dependent pure red cell anemia (PRCA) [4]. PVB19-related anemia can be resolved by treatment with intravenous immunoglobulin (Ig) [5]. However, this treatment often has a transient beneficial effect, and AIDS patients might experience a relapse of anemia. Therefore, AIDS patients may require periodic administration of intravenous Ig or blood transfusions. In recent years, some reports have shown that complete remission of PVB19-associated PRCA can be achieved by treating patients with HAART [6–8]. Although patients with HIV-1 infection show high seroprevalence of PVB19 [9], few reports have been published on primary or persistent PVB19 infection, particularly PVB19-related IRIS, in HIV-1-infected patients. In this report, we describe the case of a man with AIDS who presented with chronic PVB19-induced PRCA and IRIS after undergoing HAART. We focus on the relationship between the clinical presentation and immunological status in this condition.

Case report

A 54-year-old HIV-1-positive man visited our hospital in May 2006. He had been diagnosed with *Pneumocystis jiroveci* pneumonia and treated with sulfamethoxazole/trimethoprim in February 2006. His initial CD4 cell count was 35 cells/μl, and the plasma HIV-1 RNA level was 250,000 copies/ml. The results of other laboratory analyses were normal, except for the presence of slight anemia (hemoglobin level 11.5 g/dl). He reported that he had traveled abroad to Southeast Asia for personal reasons.

In November 2006, he re-visited our hospital, and his hemoglobin level had decreased to 7.7 g/dl. He did not show any other symptoms, such as fever, rash, or arthralgia, or any signs of cardiac, renal, or hepatic disorders. He did not report any direct contact with patients having erythema infectiosum. Two weeks later, he experienced dyspnea and was hospitalized immediately. Severe anemia was detected (hemoglobin 5.3 g/dl), and blood transfusions were performed (Fig. 1; Table 1). Gastrointestinal bleeding and hemolytic anemia were ruled out. PVB19 infection was suspected, and an immunoassay [Parvo B19 IgM-enzyme immunoassay (EIA); "SEIKEN," Denka Seiken, Tokyo, Japan] revealed anti-PVB19 IgM antibodies in the serum.

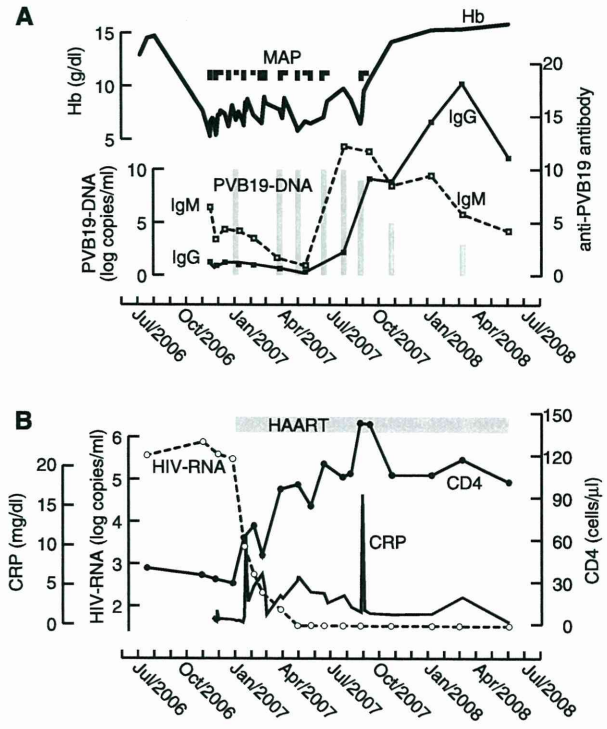

Fig. 1 The patient's clinical course with the changes in the hemoglobin levels and immunological status at the primary and persistent PVB19 infection and at the resolution of PRCA. **a** The *upper solid line* shows the time course of changes in the hemoglobin (Hb) level. The *closed boxes* indicate transfusion of 2 U of packed red blood cells. The *lower solid line* and the *broken line* represent the EIA indices of anti-PVB19 IgG and IgM antibodies, respectively. The *gray bars* show the concentration of serum PVB19-DNA. **b** Time course of changes in the CD4 cell count, plasma HIV-1-RNA levels, and CRP concentrations as well as the duration of HAART are shown

A qualitative polymerase chain reaction (PCR) analysis performed at BML Inc. (Tokyo, Japan) revealed the presence of erythrovirus DNA in the serum. The commercial assays for PVB19-DNA can detect erythrovirus DNA, including the DNA of the newly described erythrovirus variants (genotype 2 and 3) [2]. Quantitative assessment, i.e., real-time PCR analysis, was not performed at this point. The anti-PVB19 IgG antibody index assessed using Parvo B19 IgG-EIA (Seiken, Denak Seiken) was borderline positive (0.92). Examination of a bone marrow aspirate revealed an aplastic marrow (myeloid/erythroid ratio 63:1). Neither parasites nor hemophagocytic cells were found in the aspirate. Although typical giant proerythroblasts were not observed, acute PRCA caused by primary PVB19 infection was diagnosed. The patient was transfused with 6–8 U of blood per month. The anti-PVB19 IgG antibody index gradually reduced and changed from borderline-positive to negative, and the anemia did not improve; these findings indicated a transition from primary PVB19 infection to chronic and persistent infection. Intravenous Ig