Table 4. Multivariate analysis to estimate the association of SNPs of the UGT1A-3′-UTR with atazanavir-induced nephrolithiasis

UGT1A-3′-UTR	Adjusted OR	95% CI	*P* value
Genotype T/C versus C/C at position c.211	3.7	1.13–11.9	0.030
Genotype G/C versus C/C at position 339	5.8	1.56–21.3	0.009
Genotype G/G or G/C versus C/C at position 440	5.8	1.56–21.3	0.009

Each SNP was tested in the model separately.
Each variable was adjusted for sex, age and hepatitis C infection.

et al.[32] reported that these SNPs are associated with inter-individual variability in acetaminophen (paracetamol) glucuronidation in the human liver, and provide protection against acute liver failure by acetaminophen overdose, probably through more extensive detoxification of acetaminophen via glucuronidation. Because the biotransformation pathways of atazanavir or its metabolites also include glucuronidation,[12] the UGT1A-3′-UTR variants could alter atazanavir metabolism and pharmacokinetics, resulting in increased atazanavir concentration in the blood and increased excretion in urine, facilitating nephrolithiasis formation. Unfortunately, serum and urine concentrations of atazanavir were not measured in the present study. It is also notable that the *UGT1* subfamily has a unique gene structure; the *UGT1* gene has 13 exon 1s from *UGT1A1* to *UGT1A13P*, and exons 2–5, which are common in all mRNAs expressed from the gene.[36] The UGT1A-3′-UTR is located in exon 5, which is commonly present in the UGT1 subfamily (Figure 1), and thus the variants in the UGT1A-3′-UTR might influence not only UGT1A1 but also other UGT1 isoforms that take part in glucuronidation of various substrates,[36] and they might affect atazanavir metabolism and pharmacokinetics as well. Figure 1 also shows that the identified SNPs in the UGT1 3′-UTR are in tight linkage disequilibrium with the gene next to them (*HEATR7B1*), suggesting that the latter could also affect atazanavir metabolism/transportation. To our knowledge, however, there is no information on the role of *HEATR7B1* in drug metabolism/transportation, and the above conjecture remains to be investigated.

In this study, the median serum total bilirubin level in the case patients was higher than that in the control group. Rockwood *et al.*[8] reported a close relationship between hyperbilirubinaemia and the development of atazanavir-induced renal stones. However, no such relationship was found in our previous cohort study.[6] In two pharmacokinetics studies, Rodríguez-Nóvoa *et al.*[20,29] reported that serum bilirubin level correlated with plasma atazanavir concentration, and one can speculate that high bilirubin levels might reflect higher atazanavir concentrations, which result in precipitation of atazanavir in urine and renal stone formation. However, these results are still preliminary and further studies are needed to determine the true relationship between serum bilirubin level and atazanavir-related nephrolithiasis.

Several limitations of this study need to be acknowledged. First, and importantly, although this study identified association between the UGT1A-3′-UTR variants and atazanavir-induced nephrolithiasis, the number of enrolled patients was small in this case–control study; the results need to be interpreted with caution. The results could provide the basis for an exploratory hypothesis and further larger studies are needed to confirm such an association. Second, not all polymorphisms in genes of the targeted proteins were examined. Thus, we might have missed other important SNPs associated with or affecting the metabolism or transportation of atazanavir. There might be other, unknown proteins that take part in the metabolism or transportation of atazanavir that also contribute to susceptibility to atazanavir-induced nephrolithiasis. Third, because renal stone formation occurs as a composite of various factors and the components of nephrolithiasis were not analysed in the study, it is difficult to exclude the effects of classic risk factors for renal stone formation, apart from the genetic factors identified in the present study. However, the two study samples were well matched in terms of risk factors, such as BMI, serum uric acid and history of indinavir use.[4,5,24–26] Furthermore, the susceptibility to nephrolithiasis in patients on an atazanavir/ritonavir-containing regimen is well established; the incidence of nephrolithiasis is 10- to 20-fold higher in patients on atazanavir/ritonavir-containing ART than in patients on other protease inhibitor-containing ART regimens.[6,7] Fourth, because functional data are not yet available, clinical or biochemical studies to confirm the results obtained here are certainly needed. We did not measure atazanavir concentration in blood or urine.

In conclusion, in a setting where other predisposing factors for nephrolithiasis were well matched, the present study demonstrates that the Japanese HIV-1-infected patients who developed atazanavir-induced nephrolithiasis were ~5-fold more likely to have variants in the UGT1A-3′-UTR compared with those without nephrolithiasis. Further studies are warranted to confirm this association and to elucidate how these SNPs might influence the metabolism and excretion of atazanavir and the formation of nephrolithiasis.

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Short Report: Asymptomatic Intestinal Amebiasis in Japanese HIV-1–Infected Individuals

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Abstract. Seventy-one asymptomatic human immunodeficiency virus-1 (HIV-1) -infected individuals who underwent colonoscopy for detection of diseases other than amebiasis were included in this study. Ulcerative lesions caused by *Entamoeba histolytica* were identified by colonoscopy and biopsy in 11.3% (8 of 71) of individuals. Stool microscopic examination hardly identified *Entamoeba*, whereas serum antibody against *E. histolytica* was often elevated in patients with subclinical intestinal amebiasis. Human leukocyte antigen (HLA) class II allele against *E. histolytica* infection (DQB1*06:01) was frequently identified in these patients. This study emphasizes the endemic nature of *E. histolytica* infection in our cohort and the difficulties in epidemiological control.

INTRODUCTION

Invasive amebiasis caused by *Entamoeba histolytica* is the second most common cause of parasite infection-related mortality worldwide, accounting for 40,600 to 73,800 deaths annually.[1] Recent studies indicated that invasive amebiasis is prevalent in not only developing countries, where food or water is contaminated with stool, but also, East Asian developed countries, including Japan, as a sexually transmitted infection.[2–5] We reported previously high seropositivity for *E. histolytica* among asymptomatic human immunodeficiency virus-1 (HIV-1)-infected individuals in Japan and showed relatively high incidence of invasive amebiasis in that population, probably because of exacerbation of subclinical infection.[6] Other groups also reported that serum antibody against *E. histolytica* can be elevated, even in asymptomatic-infected individuals, and that seroconversion was seen in the absence of any symptoms in longitudinal follow-up in endemic areas.[7] These results indicate that subclinical infection of *E. histolytica* is frequent in high-risk populations, making it difficult to control *E. histolytica* endemicity.

Evidence suggests that human leukocyte antigen (HLA) type plays a role in amebiasis. For example, Duggal and others[8] reported previously that HLA DQB1*0601 seemed to provide protection against *E. histolytica* infection in Bangladeshi children.

This cross-sectional study was designed to determine the prevalence of ulcerative lesions associated with *E. histolytica* infection in asymptomatic HIV-1–infected individuals in Japan. We also examined the pathogenesis of subclinical intestinal amebiasis and the role of HLA genotypes.

MATERIALS AND METHODS

Ethics statement. The study was approved by the Human Research Ethics Committee of our hospital, the National Center for Global Health and Medicine in Tokyo. The study was conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all participants. No children were included in the study.

Study design and participants. This cross-sectional study included HIV-infected patients who underwent colonoscopy between June of 2010 and June of 2013. One week before colonoscopy, each patient filled out a questionnaire about lower gastrointestinal symptoms based on the Gastrointestinal Symptom Rating Scale (GSRS) rating on a seven-graded Likert scale.[9] Asymptomate for lower gastrointestinal diseases was defined as GSRS scores of one or two for three questions on the diarrhea syndrome domain (diarrhea, loose stools, and urgent need to defecate) and one question on bloody stool.[10] Serum antibody testing against *E. histolytica* was performed in all participants on the day of colonoscopy. Serum antibody was tested by indirect fluorescent antibody assay using whole *E. histolytica* antigen according to the protocol described in the instruction sheet of the approved kit (bioMerieux, SA). Seropositivity was defined as positive response in a serum sample diluted at 1:100 ($\times 100$), and anti-Eh titer was determined by the highest dilution for the positive response. HLA type was determined by standard sequence-based genotyping (HLA Laboratory, Kyoto, Japan). The diagnosis of subclinical intestinal infection of *E. histolytica* was established on confirmation of one or two of the following two criteria: (1) identification of amebic trophozoites in biopsy specimens from gross ulcerative lesions obtained during colonoscopy and/or (2) no pathogens identified in biopsy specimens of gross ulcerative lesion, which were compatible with amebic ulcer,[11] but ulcerative lesion resolved completely after metronidazole monotherapy as confirmed by colonoscopy.

Statistical analysis. The patients' characteristics and serum positivities for anti-*E. histolytica* antibody were compared using χ^2 or Mann–Whitney U test for qualitative or quantitative variables, respectively. Statistical significance was defined as two-sided P value < 0.05. All statistical analyses were performed using The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL).

RESULTS

Study population. In total, 380 HIV-1–infected individuals were enrolled during the study period, and 71 patients met the criteria of no symptoms for lower gastrointestinal diseases according to the GSRS. The most common reason for colonoscopy was colorectal cancer screening ($N = 48$), whereas

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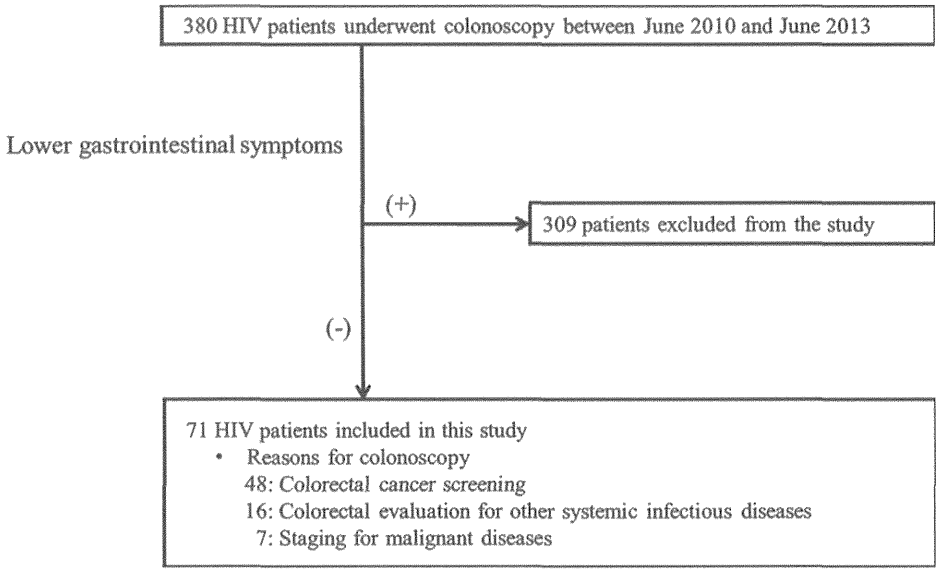

FIGURE 1. Flow diagram of the patient recruitment process. Lower abdominal symptoms were collected based on the GSRS rating on a seven-graded Likert scale at 1 week before colonoscopy.

the other 23 patients underwent colonoscopy for evaluation of progression of malignancies or infections (e.g., malignant lymphoma, Kaposi's sarcoma, tuberculosis, and cytomegalovirus) (Figure 1).

Frequency of intestinal amebic infection among asymptomatic HIV-1–infected individuals. Amebic colitis was confirmed in eight (11.3%) cases. Gross ulcerative lesions were identified by colonoscopy in all eight cases. Amebic trophozoite was identified in the biopsy specimens of five cases (Figure 2). Although amebic trophozoites were not identified in the biopsy specimens of the other three cases, their sera were positive for antibody against *E. histolytica*. In all patients, the ulcerative lesions resolved completely after metronidazole monotherapy.

Clinical features and presentation of patients with and without intestinal amebic infection. As shown in Table 1, patients with amebic intestinal ulcerative lesions tended to be younger, be male homosexuals, have low CD4 counts, and have high HIV-RNA levels, although these differences were not statistically significant. Multiple ulcerative lesions were found in four cases (50%), and the most frequently involved location was the cecum (five cases; 62.5%). Serum antibody against *E. histolytica* was positive in 7 of 8 (87.5%) patients with amebic intestinal ulcerative lesions compared with positivity in only 11 of 63 (17.5%) patients without amebic ulcerative lesions (Table 2).

From the limited data on fecal occult blood testing (FOB) and stool microscopic examination before treatment in cases with amebic ulcerative lesions, FOB was positive in two of three cases (66.7%), and the cyst form, not trophozoite form, *Entoamoeba* was found in only one of four cases (25%).

HLA class II allele frequencies in patients with and without subclinical intestinal amebiasis. HLA data were available for 57 patients (7 of 8 patients with amebiasis and 50 of 63 patients without amebiasis) in our study. We investigated the relation between HLA alleles identified in more than five patients (frequency > 10%) and subclinical intestinal amebiasis. HLA DQB1*06:01 allele was significantly more frequent in patients with subclinical intestinal amebiasis than those without it (Table 3). All the HLA DQB1*06:01 holders were heterozygotes. The frequency of the HLA DRB1*15:02 allele was also significantly higher in patients with subclinical intestinal amebiasis ($P = 0.05$); 7 of 10 patients with HLA DQB1*06:01 also held HLA DRB1*15:02. No colonic amebic ulceration was detected in DQB1*06:01 (–)/DRB1*15:02 (+) patients. Thus, DQB1*06:01 seemed to be the primary HLA allele associated with subclinical intestinal amebiasis in the study population.

DISCUSSION

The pathogenesis of amebiasis remains unclear, including the incubation period after cyst ingestion and the mechanism of spontaneous remission. We reported previously high seroprevalence of *E. histolytica* (21.3%) in HIV-1–infected individuals and that the majority of these patients (78.3%) had no history of invasive amebiasis. In that study, the patients were considered to be at high risk for developing symptomatic amebic infection in longitudinal follow-up (about 20% within the first 1 year of the follow-up period).[6] Based on those results, we speculated the presence of subclinical intestinal amebiasis in patients positive for antibody against *E. histolytica* in the serum resulting in high frequency of symptomatic amebic diseases thereafter, although we did not identify the lesions of *E. histolytica* in these individuals in that study. However, Okamoto and others[12] reported that intestinal ulcerative lesions of *E. histolytica* were rare based on colonoscopic examination in the general population in Japan with positive FOB (0.1%; 4 of 5,193). Our group reported previously that patients with cecal amebic ulcers were sometimes asymptomatic.[11] In this regard, however, the clinical significance of *E. histolytica* infection in asymptomatic individuals had not been fully assessed. In this study, we identified gross amebic ulcers by colonoscopy in 11.2% of asymptomatic HIV-1–infected individuals.

Detection of intestinal amebiasis in asymptomatic individuals is important for not only treatment but also, epidemiological control, especially in endemic areas, because individuals

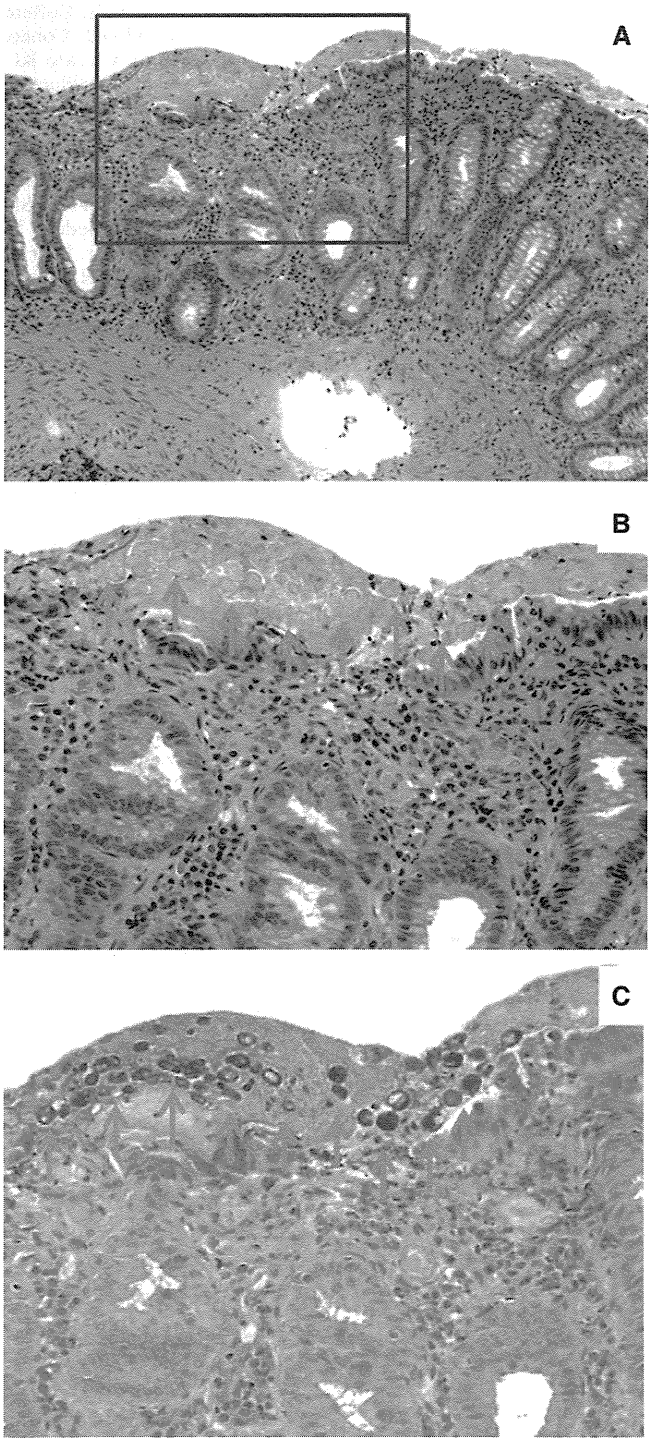

FIGURE 2. Histopathological findings in subclinical intestinal amebiasis. Colonic tissue section was obtained during colonoscopy from a representative asymptomatic patient. *E. histolytica* on the surface of large-intestinal mucosa was clearly stained with periodic acid-Schiff (PAS) staining (green arrows). (**A**) Hematoxylin-eosin staining, ×100. (**B**) Higher magnification of the boxed area in **A**. Hematoxylin-eosin staining, ×400. (**C**) PAS staining, ×400.

with intestinal amebic ulcers can act as a reservoir for *E. histolytica*. However, it is sometimes difficult to identify amebiasis in these individuals, because they lack typical abdominal symptoms related to amebiasis, such as tenesmus, diarrhea, and dysentery. Moreover, our results showed that stool microscopic examination hardly identified amebiasis in these individuals. FOB is more sensitive than stool microscopic examination. However, FOB was positive in 72.7% (16 of 22) of patients free of amebic ulceration. Serum antibody against *E. histolytica* might be a sensitive marker of amebic ulcer in asymptomatic individuals. However, low titers of serum antibody were frequently found in individuals without amebic ulcer. The optimal cutoff value of antibody titer for amebic ulcer is still unclear (for cutoff titer of ×100, sensitivity is 87.5%, and specificity is 82.5%, whereas for cutoff titer ×400, sensitivity is 75.0%, and specificity is 95.2%) (Table 2).

Interestingly, our analysis showed high frequency of HLA DQB1*06:01 heterozygote in patients with subclinical intestinal amebiasis. This allele was reported previously to provide protection against *E. histolytica* infection in Bangladeshi patients.[8] One possible explanation is that ulcerative lesions could occur asymptomatically in patients with HLA DQB1*06:01 and that their immune system could prevent the development of invasive disease from *E. histolytica*, resulting in the high frequency of subclinical intestinal amebiasis observed in our cross-sectional analysis. Genetic differences between Bangladeshi and Japanese patients should also be considered. HLA DQB1*06:01 and DRB1*15:01 were the most common haplotypes in Bangladesh, although they were not identified in our patients.

TABLE 1
Characteristics of patients with and without subclinical intestinal amebiasis

	Amebiasis	No amebiasis	*P* value
n	8	63	
Age (years), median (range)	39 (27–62)	51 (26–81)	0.07
Male sex (%)	8/8 (100%)	56/63 (88.9%)	1.00
Men who have sex with men (%)	8/8 (100%)	44/63 (69.8%)	0.10
Past history of amebiasis (%)	0/8 (0%)	7/63 (11.1%)	1.00
CD4/μL, median (range)	301 (70–584)	436 (21–1,697)	0.28
HIV-RNA (LC/mL), median (range)	4.02 (UD–5.41)	UD (UD–5.85)	0.09

LC/mL = log 10 copies per milliliter; UD = undetectable.

TABLE 2
Clinical presentation of patients with and without subclinical intestinal amebiasis

	Amebiasis	No amebiasis	*P* value
n	8	63	
Serum positivity for anti-*E. histolytica* antibody (%)	7/8 (87.5%)	11/63 (17.5%)	< 0.001
< ×100	1	52	
×100	1	5	
×200	0	3	
×400	3	2	
×800	1	1	
×1,600	2	0	
Site of intestinal amebiasis			
Cecum	5		
Ascending	3		
Transverse	1		
Descending	0		
Sigmoid	1		
Rectum	4		

TABLE 3
Frequencies of HLA class II alleles in patients with and without amebiasis

	Patients with amebiasis ($N = 7$)	Patients without amebiasis ($N = 50$)	P value
DRB1			
*04:03	1 (14.3%)	5 (10.0%)	0.56
*04:05	3 (42.9%)	16 (32.0%)	0.68
*04:06	1 (14.3%)	5 (10.0%)	0.56
*09:01	1 (14.3%)	17 (34.0%)	0.41
*11:01	0 (0.0%)	6 (12.0%)	1.00
*13:02	0 (0.0%)	7 (14.0%)	0.58
*15:01	1 (14.3%)	7 (14.0%)	1.00
*15:02	3 (42.9%)	5 (10.0%)	0.050
DQB1			
*03:01	1 (14.3%)	11 (22.0%)	1.00
*03:02	2 (28.6%)	12 (24.0%)	1.00
*03:03	1 (14.3%)	20 (40.0%)	0.24
*04:01	3 (42.9%)	16 (32.0%)	0.68
*05:02	1 (14.3%)	3 (6.0%)	0.42
*05:03	0 (0.0%)	6 (12.0%)	1.00
*06:01	5 (71.4%)	5 (10.0%)	0.001
*06:02	1 (14.3%)	7 (14.0%)	1.00
*06:04	0 (0.0%)	7 (14.0%)	0.58

Data are numbers and frequencies of patients harboring each HLA allele. HLA data were available in 57 patients. HLA alleles identified in more than five patients (> 10%) were considered.

Additional studies are needed to examine the effects of host genetic factors on *E. histolytica* infection and the development of invasive disease. Interestingly, not only HLA but also, mutation of the leptin receptor were reported to be associated with amebic infection.[13]

In conclusion, intestinal amebic ulcerative lesions were frequently found in asymptomatic HIV-1–infected Japanese individuals who could otherwise act as reservoirs for new infection in other high-risk populations. Additional studies of subclinical infection are needed to control the *E. histolytica* endemicity.

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Brain Magnetic Resonance Imaging Screening Is Not Useful for HIV-1-Infected Patients Without Neurological Symptoms

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Abstract

We investigated the diagnostic usefulness of brain magnetic resonance imaging (MRI) screening in HIV-1-infected patients without neurological symptoms in detecting intracranial diseases at early stages. In this retrospective analysis, the study patients were HIV-1-infected patients who underwent brain MRI scan in clinical practice between 2001 and 2013. We excluded patients with MRI for (1) follow-up examination for prediagnosed intracranial diseases, (2) cancer staging, (3) screening mycobacterium/bacteria/fungi disease proliferation in the brain, and (4) evaluation for meningitis/encephalitis. The study patients ($n=485$) were classified into two groups: those who underwent brain MRI scan without any neurological symptoms/signs (asymptomatic patients, $n=158$) and those who underwent MRI due to such symptoms (symptomatic patients, $n=327$). Asymptomatic patients had lower CD4 counts than symptomatic patients (median 78 versus 241/μl). Intracranial diseases were detected in three (2%) of the asymptomatic patients [two toxoplasmosis and one progressive multifocal leukoencephalopathy (PML)] compared to 58 (19%) of the symptomatic patients (the χ^2 test, $p<0.01$). The latter included toxoplasmosis ($n=10$), PML ($n=7$), cytomegalovirus encephalitis ($n=3$), primary central nervous system lymphoma ($n=3$), cryptococcoma/meningitis ($n=3$), and HIV-associated dementia ($n=17$). Among symptomatic patients, intracranial diseases were common in those with slurred speech (3/6, 50%), seizure (4/10, 40%), eyesight/vision abnormality (5/16, 31%), altered mental status (8/31, 26%), and hemiplegia/numbness (13/50, 26%). For patients with CD4 count $<200/\mu$l, intracranial diseases were detected in only 3 (3%) of 144 asymptomatic patients, compared with 46 (32%) of 113 symptomatic patients ($p<0.01$). Brain MRI screening for HIV-1-infected patients without neurological symptoms is of little value.

Introduction

Patients with advanced HIV-1 infection are prone to develop intracranial opportunistic diseases, such as toxoplasma encephalitis, primary central nervous system lymphoma (PCNSL), progressive multifocal leukoencephalopathy (PML), and cytomegalovirus (CMV) encephalitis.[1] Although the introduction of antiretroviral therapy (ART) substantially decreased the incidence of neurological opportunistic infections,[2,3] such diseases have high associated mortality even with appropriate treatment, and recurrences and residual neurological deficits can occur.[4,5] Because delayed diagnosis of these intracranial diseases has a detrimental effect on patients with HIV-1 infection,[5,6] early diagnosis, not to mention prevention, of such diseases is of importance.

Brain magnetic resonance imaging (MRI) is often preferred to computed tomography (CT) in establishing the diagnosis of many of these diseases due to its superior sensitivity to subtle white matter and meningeal disease.[7–10] However, there is no information on the utility of brain MRI screening for HIV-1-infected patients without neurological symptoms/signs in detecting intracranial opportunistic diseases at early stages. This observational study was designed to assess the usefulness of brain MRI screening of such patients with HIV-1 infection.

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Materials and Methods

Study design, setting, and participants

We conducted an observational single-center study to investigate the usefulness of brain MRI screening in HIV-1-infected patients without neurological symptoms who warrant investigation for intracranial diseases. The study was conducted at the AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo, the largest referral center for HIV care in Japan.[11] The study patients were those who fulfilled the following inclusion criteria: HIV-1-infected patients who underwent brain MRI scan in clinical practice between June 2001 and August 2013. In addition, the following exclusion criteria were applied: patients who underwent brain MRI for (1) follow-up examination during the study period because of intracranial diseases such as opportunistic infections, stroke, or malignancy, which were diagnosed prior to the referral to our clinic, (2) staging of malignant tumors, (3) screening mycobacterium/bacteria/fungi disease proliferation in the brain in patients who were already diagnosed with mycobacterial diseases or bacteremia or fungemia, and (4) evaluation of meningitis/encephalitis.

The study patients ($n=485$) were classified into those who underwent brain MRI scan without any neurological symptoms, such as seizure, altered mental status, hemiplegia/numbness, headache, or fever (asymptomatic patients, $n=158$), and those who underwent MRI due to the abovementioned symptoms, which can suggest a focal brain lesion[5] (symptomatic patients, $n=327$). Asymptomatic patients included those who underwent MRI due to positive antitoxoplasma IgG antibody ($n=38$) and positive serum cryptococcal antigen ($n=1$). At our clinic, patients with a low CD4 cell count (typically less than 200/μl) often underwent brain MRI even though they had no neurological symptoms/signs that would warrant a brain imaging examination to rule out intracranial opportunistic infections or malignancy at early stages.

The study was approved by the Human Research Ethics Committee of NCGM. All patients included in this study provided written informed consent for their clinical and laboratory data to be used and published for research purposes. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Measurements

At our hospital, brain MRI was routinely read by one experienced radiologist and the findings were confirmed by another radiologist. Furthermore, the MRI diagnosis was confirmed by reviewing the medical records and follow-up brain imaging when available. The diagnostic criteria for cryptococcal meningitis, cytomegalovirus encephalitis, and toxoplasmic encephalitis were those adopted by the AIDS Clinical Trials Group (ACTG)-A5164.[12] HIV-associated dementia in this study was diagnosed based on the MRI findings, which included generalized atrophy and prominent white matter changes plus cognitive impairment based on the chart review, and not necessarily required neurocognitive function tests.[8] The reasons for conducting an MRI were also extracted from the medical records. Baseline characteristics and HIV-1-related variables at the time of brain MRI were also extracted from the medical records. They included age, sex, ethnicity, history of AIDS, route of HIV-1 transmission, treatment status for HIV-1 infection (either treatment naive or experienced), CD4 cell count, and HIV viral load. For CD4 count and HIV load, we used data collected closest to and preceding by up to 3 months the day of the brain MRI. In Japan, because the prescription period under the health care system is limited to 3 months, patients need to visit the HIV Clinic at least once every 3 months for prescriptions as well as monitoring CD4 cell count and HIV-1 load.[11]

Statistical analysis

Baseline characteristics were compared between asymptomatic and symptomatic patients using the Student's t-test and χ^2 test (Fisher's exact test) for continuous and categorical variables, respectively. Prevalence of intracranial diseases was calculated among asymptomatic patients and compared to that of symptomatic patients with the χ^2 test. The logistic regression model was used to estimate the associations of lack of neurological symptoms/signs over the presence of such symptoms/signs with the MRI findings of intracranial diseases. The model was adjusted for age, sex, CD4 count, HIV treatment status, and history of AIDS. Subgroup analysis included the prevalence of intracranial diseases in patients with a CD4 count $<200/\mu$l. Statistical significance was defined as two-sided p values <0.05. We used odds ratios (ORs) with 95% confidence intervals (95% CIs). All statistical analyses were performed with The Statistical Package for Social Sciences ver. 21.0 (SPSS, Chicago, IL).

Results

The study included 485 patients who underwent a brain MRI scan in clinical practice, of whom 158 had no neurological symptoms (asymptomatic) and 327 did have such symptoms (symptomatic). Of the total patients, 475 (98%) were Asians, 446 (92%) were males, and 365 (75%) were infected with HIV-1 through homosexual contact (Table 1). The median age of the study patients was 41 [interquartile range (IQR) 34–51]. Asymptomatic patients had a lower CD4 count [median 78/μl, interquartile range (IQR) 21–237, symptomatic: 241/μl, 60–470 ($p<0.01$)] and higher HIV-1 viral load [4.84 $\log_{10}$/ml, IQR 2.97–5.62, symptomatic: 2.95 $\log_{10}$/ml, 1.70–5.11 ($p<0.01$)] than symptomatic patients. Asymptomatic patients were more likely to be treatment naive (68% versus 41%, $p<0.01$) and have a history of AIDS (62% versus 47%, $p<0.01$). There was no significant difference in other baseline characteristics between the two groups (Table 1).

Among the 158 asymptomatic patients, brain MRI screening detected toxoplasmosis ($n=2$) and PML ($n=1$, with CD4 43/μl), i.e., a prevalence of intracranial diseases of 2%. The two patients with toxoplasmosis underwent brain MRI due to positive antitoxoplasma IgG antibody with a titer of 20,480 (CD4 168/μl) and 1,280 (CD4 16/μl) IU/ml. In asymptomatic patients who underwent brain MRI due to positive antitoxoplasma IgG antibody, intracranial diseases were detected in 3 (8%) out of 38 patients (Table 2). On the other hand, brain MRI for symptomatic patients detected 58 intracranial diseases with a prevalence of 19%. The cases included toxoplasmic encephalitis ($n=10$), PML ($n=7$), CMV encephalitis ($n=3$), PCNSL ($n=3$), cryptococcosis/meningitis ($n=3$), herpes simplex virus encephalitis ($n=1$), HIV-associated dementia ($n=17$), acute cerebral infarction ($n=8$), gummatous

TABLE 1. CLINICAL CHARACTERISTICS OF THE STUDY PATIENTS ACCORDING TO NEUROLOGICAL SYMPTOMS

	All patients (n=*485*)	*Patients without neurological symptoms* (n=*158*)	*Patients with neurological symptoms* (n=*327*)	p *value*
Male sex, *n* (%)	446 (92)	146 (92)	300 (92)	0.86
Age[†]	41 (34–51)	42 (33–52)	41 (35–49)	0.95
Asian, *n* (%)	475 (98)	154 (98)	321 (98)	0.74
CD4 cell count (/μl)[a]	178 (41–420)	78 (21–237)	241 (60–470)	<0.01
HIV-1 load ($\log_{10}$/ml)[a]	4.20 (1.70–5.26)	4.84 (2.97–5.61)	2.95 (1.70–5.11)[b]	<0.01
Homosexual contact, *n* (%)	364 (75)	117 (74)	247 (76)	0.74
Treatment naive, *n* (%)	240 (50)	107 (68)	133 (41)	<0.01
History of AIDS, *n* (%)	250 (52)	98 (62)	152 (47)	<0.01

[a]Median (interquartile range).
[b]Data on HIV-1 load are not available for two patients.

syphilis ($n=1$), tuberculoma ($n=1$), metastatic cancer ($n=1$), chronic subdural hematoma ($n=1$), schwannoma ($n=1$), and progressive supranuclear palsy ($n=1$) (Table 2). In asymptomatic patients, intracranial diseases were less likely to be detected by brain MRI, compared to symptomatic patients [by univariate and multivariate analysis (OR=0.1; 95% CI, 0.03–0.29; $p<0.01$) (adjusted OR=0.1; 95% CI, 0.02–0.17; $p<0.01$)]. Patients with higher CD4 counts were also less likely to have intracranial diseases (per 100/μl increment, adjusted OR=0.7; 95% CI, 0.55–0.83; $p<0.01$). Among the symptomatic patients, those who presented with slurred speech, seizure, eyesight/vision abnormality, altered mental status, and hemiplegia/numbness were highly likely to have intracranial diseases, with a prevalence of 50%, 40%, 31%, 26%, and 26%, respectively (Table 3).

Subgroup analysis limited to data of patients with CD4 count of <200/μl showed that the abovementioned three intracranial diseases were detected in 144 asymptomatic patients with a prevalence of 3%, compared to 46 (32%) of 113 symptomatic patients (asymptomatic over symptomatic, OR=0.1; 95% CI, 0.02–0.19; $p<0.01$) (Table 2). Only a few intracranial opportunistic diseases were diagnosed in patients with a CD4 count of ≥200/μl; PCNSL ($n=1$), HIV-associated dementia ($n=4$), acute cerebral infarction ($n=6$), metastatic cancer ($n=1$), and progressive supranuclear palsy ($n=1$).

Discussion

In this observational study of patients who underwent brain MRI screening in clinical practice, only 2% of patients without neurological symptoms/signs that warranted investigation of intracranial diseases were found to have intracranial diseases, whereas a significantly higher prevalence (19%) of intracranial diseases was detected in patients who underwent brain MRI due to such symptoms. Among patients with a CD4 count of <200/μl, who are reported to be at high risk for intracranial diseases,[5,10] the result was similar; 3% and 32% of asymptomatic and symptomatic patients, respectively, were found to have intracranial diseases. On the other hand, high detection rates of intracranial diseases by brain MRI were observed in patients who presented with slurred speech (50%), seizure (40%), eyesight/vision abnormality (31%), altered mental status (26%), and hemiplegia/

TABLE 2. PREVALENCE OF INTRACRANIAL DISEASES DETECTED BY BRAIN MAGNETIC RESONANCE IMAGING ACCORDING TO NEUROLOGICAL SYMPTOMS

Intracranial diseases	*Patients without neurological symptoms* (n=*158*)	*Patients without neurological symptoms with CD4 <200/*μl (n=*144*)	*Patients with neurological symptoms* (n=*327*)	*Patients with neurological symptoms with CD4 <200/*μl (n=*113*)	*Positive toxoplasma Ab and without neurological symptoms* (n=*38*)
Toxoplasmosis	2 (1)	2 (2)	10 (3)	10 (7)	2 (1)
PML	1 (1)	1 (1)	7 (2)	7 (5)	1 (1)
HIV-associated dementia			17 (6)	13 (9)	
Malignant lymphoma			4 (1)	3 (2)	
CMV encephalopathy			3 (1)	3 (2)	
Cryptococcoma/meningitis			3 (1)	3 (1)	
HSV encephalopathy			1	1	
Gummatous syphilis			1	1	
Tuberculoma			1	1	
Metastatic cancer			1		
Cerebral infarction			8 (3)	2 (1)	
Others			3 (1)	2 (1)	
Total	3 (2)	3 (3)	59 (19)	46 (32)	3 (8)

Data are numbers (percentages) of patients.
Ab, antibody; PML, progressive multifocal leukoencephalopathy; CMV, cytomegalovirus; HSV, herpes simplex virus.

TABLE 3. PREVALENCE OF INTRACRANIAL DISEASES DETECTED BY BRAIN MAGNETIC RESONANCE IMAGING ACCORDING TO NEUROLOGICAL SYMPTOM CATEGORIES

	Intracranial diseases	*Prevalence of intracranial diseases*
Slurred speech ($n=6$)	Cerebral infarction $n=2$ PML $n=1$	50%
Seizure ($n=10$)	Toxoplasmosis $n=2$ PML $n=1$ HSV encephalitis $n=1$	40%
Eyesight/vision abnormality ($n=16$)	Malignant lymphoma $n=2$ HIV-associated dementia $n=2$ Metastatic cancer $n=1$	31%
Altered mental status ($n=31$)	Toxoplasmosis $n=2$ HIV-associated dementia $n=2$ Cryptococcoma/meningitis $n=2$ PML $n=1$ Tuberculoma $n=1$	26%
Hemiplegia/numbness ($n=50$)	Cerebral infarction $n=5$ Toxoplasmosis $n=3$ PML $n=3$ HIV-associated dementia $n=1$ Other $n=1$	26%
Neurocognitive impairment ($n=62$)	HIV-associated dementia $n=9$ Cerebral infarction $n=1$ CMV encephalitis $n=2$	19%
Fever work-up ($n=12$)	Malignant lymphoma $n=1$ HIV-associated dementia $n=1$	17%
Dizziness/vertigo/tinnitus ($n=45$)	Toxoplasmosis $n=1$ PML $n=1$ Malignant lymphoma $n=1$ HIV-associated dementia $n=1$ CMV encephalitis $n=1$	11%
Abnormal ophthalmologic examination ($n=11$)	HIV-associated dementia $n=1$	9%
Headache ($n=49$)	Toxoplasmosis $n=2$	4%
Syncope ($n=16$)		0%

PML, progressive multifocal leukoencephalopathy; HSV, herpes simplex virus; CMV, cytomegalovirus.

numbness (26%). The present study indicates that brain MRI screening for HIV-1-infected patients without neurological symptoms/signs, even those with a low CD4 count (< 200/μl), is of little value. In contrast, MRI screening is useful for patients with particular neurological symptoms/signs. These findings can help reduce unnecessary brain MRI examinations and can be helpful in clinical decision making.

Interestingly, in both of the two asymptomatic toxoplasmic encephalitis patients who underwent brain MRI screening because of positive antitoxoplasma IgG antibody, the antibody titer was very high (20,480 IU/ml and 1,280). Together with the fact that the prevalence of intracranial diseases in asymptomatic patients with positive antitoxoplasma IgG antibody was higher (8%) than the 2% in the entire group of asymptomatic patients, brain MRI screening for patients without neurological symptoms/signs who presented with high antitoxoplasma antibody may be of value and clinically justifiable.

Our study has certain limitations. First, because brain MRI was performed at the discretion of the treating physician, patient selection bias, especially among those without neurological symptoms/signs, cannot be ruled out. However, we had a large number of study patients, and considering the availability and cost of an MRI scan, the results of the present study are of value and are useful in clinical decision making. Second, because endemic opportunistic infections vary depending on the region[13,14] and the majority of our patients were Asian, the results of the present study might not be applicable to patients in other regions. Third, in this study the diagnosis of HIV-associated dementia was based on the MRI findings plus cognitive impairment based on a chart review, and the patients did not necessarily undergo neurocognitive function tests.[8] This is because the present study included patients from 2001, long before the diagnostic Frascati criteria for an HIV-associated neurocognitive disorder that required neurocognitive function tests were established.[15]

In conclusion, although our results suggest that brain MRI screening is of little value in HIV-1-infected patients without neurological symptoms/signs that warrant investigation on intracranial diseases, it should be performed in HIV-1-infected patients who present with particular neurological symptoms, such as slurred speech and seizure.

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Original article

Low body weight and tenofovir use are risk factors for renal dysfunction in Vietnamese HIV-infected patients. A prospective 18-month observation study

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Low body weight

ABSTRACT

Background: The use of tenofovir has been rapidly increasing in Vietnam. Several studies identified low body weight as a risk factor for tenofovir-induced nephrotoxicity. However, little is known about the impact of tenofovir on renal function in HIV-infected Vietnamese with generally low weight.
Methods: An observational single-center cohort of adult HIV-infected patients on antiretroviral therapy at National Hospital of Tropical Diseases, Hanoi. Patients on tenofovir or with creatinine clearance ≤60 ml/min at baseline were excluded. The incidence of renal dysfunction was compared between patients who switched to tenofovir and those who did not. Renal dysfunction was defined as 25% decline of creatinine clearance from baseline. Time to renal dysfunction was analyzed by the Kaplan–Meier method between the two groups. The Cox hazard model was used to determine risk factors for renal dysfunction in uni- and multivariate analyses.
Results: Of 556 patients enrolled in this study, 403 were non-tenofovir group while 153 were the tenofovir-switched group. Renal dysfunction occurred at a higher rate in the tenofovir-switched group (92.5 per 1000 person-years) than the non-tenofovir group (47.8 per 1000 person-years)($p = 0.023$, Log-rank test). Multivariate analysis confirmed that tenofovir use, low body weight and glucosuria were significant risk factors for renal dysfunction (hazard ratio = 1.980; 95% confidential interval, 1.094–3.582, HR = 1.057; 95%CI, 1.016–1.098, HR = 5.202; 95%CI, 1.245–21.738, respectively).
Conclusions: Tenofovir use, low body weight and glucosuria were significant risk factors for renal dysfunction. We suggest close monitoring of renal function in patients with these risk factors even in resource-limited setting.

Key points

Treatment with TDF and low body weight were significant risk factors for renal dysfunction in Vietnamese HIV-treated patients. Given that the average body weight of Vietnamese is small, close monitoring of renal function in HIV-1-infected patients is important during treatment with TDF.

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1. Introduction

Although renal dysfunction is an important cause of morbidity and mortality in HIV-infected patients [1–7], only limited information is available on renal function in Vietnamese HIV-infected patients. Along with the 2010 WHO guidelines which phased out stavudine and recommended tenofovir (TDF) (URL: http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf), the use of TDF had been increasing in Vietnam in recent years.

TDF-associated nephrotoxicity is well known adverse effect. However, a meta-analysis study that evaluated the safety of TDF concluded that TDF-associated nephrotoxicity can be considered negligible and thus there is no need to restrict TDF use even when regular observation of renal function is not feasible [8]. Other experimental and clinical studies, however; provide a different scenario: one study of rhesus macaques described a dose-dependent nephrotoxic effect for TDF [9] and several studies reported cases of TDF-associated nephrotoxicity in low-body-weight HIV-infected patients [10,11]. Our group also reported that low body weight and use of TDF were significantly associated with chronic kidney dysfunction in Vietnamese HIV-infected patients in a cross-sectional study [12]. Since Vietnamese have a considerably smaller body weight compared with Caucasians, and the use of TDF in Vietnam is increasing throughout the country, the potential risk for TDF-related nephrotoxicity is a concern in Vietnam. This is also true in all countries in the region since the Asian population is, in general, of low body weight. To examine this issue in more detail, we conducted a longitudinal study to evaluate the incidence of renal dysfunction in Vietnamese HIV-infected patients and the risk factors of such morbidity, including use of TDF and low body weight.

2. Patients and methods

2.1. Study design

We performed a prospective observational study of a single-center cohort of Vietnamese HIV-infected patients on antiretroviral therapy (ART) to evaluate the impact of TDF and low body weight on renal function. This cohort was established in 2007 at the National Hospital of Tropical Disease (NHTD) in Hanoi, one of the biggest outpatient clinics for HIV infected-patients in Vietnam. The population of the cohort consists of Vietnamese HIV-infected patients on ART aged more than 17 years referred to NHTD.

To evaluate renal function, serum creatinine had been measured since October 2011, which is the baseline of this study. Entry criteria were patients who were registered in this cohort on October 2011. Patients taking TDF or with serum creatinine clearance (Crcl) of ≤60 ml/min at baseline were excluded. Also excluded from the study were patients whose creatinine was not obtained twice at least. The follow-up period was 18 months (between October 2011 and April 2013). All patients of this cohort received ART at baseline. ART included Zidovudine (AZT)/Lamivudine (3 TC), Stavudine (d4T)/3 TC or TDF/3 TC as nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) in combination with efavirenz (EFV), Nevirapine (NVP) or ritonavir boosted lopinavir (LPV/r). To estimate the incidence of renal dysfunction in this population, patients were divided into those who switched to TDF and those who did not. Laboratory data, including serum creatinine, were measured twice a year (in April and October) in this cohort. The study was approved by the Human Research Ethics Committee of NHTD. Each patient included in this study provided a written informed consent for the clinical and laboratory data to be used for publication. The study was conducted according to the principles expressed in the Declaration of Helsinki.

2.2. Measurements

Clinical and laboratory data included demographic variables (age, sex and weight), serum creatinine (mg/dl, measured by Jaffe method), CD4 cell count (cell/mm^3, measured by flow cytometry), plasma HIV-RNA (copies/ml, measured by the Roche COBAS TaqMan HIV monitor assay), complete history of ART, use of cotrimoxazole, date of HIV diagnosis, and presence of other comorbidities such as hepatitis B and C virus, diabetes mellitus and AIDS defining diseases. Renal dysfunction was defined as 25% decline in Crcl estimated by the Cockcroft–Gault formula, relative to the baseline.

2.3. Statistical analysis

Baseline characteristics were compared between case patients and control patients by the Student's t-test for continuous variables and by either the χ^2 test or Fisher's exact test for categorical variables. The time from baseline to renal dysfunction was analyzed by the Kaplan–Meier method for patients who switched to TDF and those who did not, and the log-rank test was used to determine the statistical significance. Censored cases represented those who died, dropped out, or were referred to other facilities before the end of follow-up period. The Cox proportional hazards regression analysis was used to estimate the impact of TDF use on the incidence of renal dysfunction. The impact of basic demographics, baseline laboratory data, and other medical conditions was also estimated with univariate Cox proportional hazards regression. Variables significantly associated with renal dysfunction in univariate analysis ($p < 0.05$) were entered into multivariate analysis. Statistical significance was defined at two-sided p value < 0.05. We used the hazard ratio (HR) and 95% confidence interval (95%CI) to estimate the association of each variable with renal dysfunction. All analyses were performed in SPSS (version 22.0).

3. Results

At baseline, 793 Vietnamese HIV-infected patients on ART were registered in this study. However, 237 patients were excluded from the study due to existing renal dysfunction at baseline (Crcl < 60 ml/min, $n = 72$), had already been treated with TDF at baseline ($n = 143$), and lack of repeated measurements of Crcl ($n = 22$). Thus, 556 patients who received ART met the study criteria and were included in the study. Of these, 153 patients were switched to TDF during the study period, while 403 patients continued treatment with non-TDF-containing regimen. The criteria for switch to TDF were adverse event caused by ART or induction of treatment for chronic hepatitis B virus infection.

Table 1 compares the baseline demographics and clinical variables of patients of the TDF-switched group and the non-TDF group. The TDF-switched group was significantly more likely to be males, hepatitis B virus S antigen-positive and hepatitis C virus antibody-positive compared to the non-TDF group. The TDF-switched group had marginally significant trend to be older and have diabetes mellitus. Body weight, serum creatinine, CD4 count, HIV RNA viral load, duration of ART, frequency of proteinuria and glucosuria, use of ritonavir boosted lopinavir (LPV/r) and cotrimoxazole, and history of AIDS-defining disease were not significantly different between the two groups. The mean CD4 count was >300/mm^3 and the mean HIV RNA load was <100 copies/ml in both groups.

During the observation period, renal dysfunction, defined as 25% decline in Crcl, was observed in 19 (12.4%) of the TDF-switched group and 27 (6.7%) of the non-TDF group, with an estimated incidence of 92.5 and 47.8 per 1000 person-years, respectively. Fig. 1 depicts the time from the baseline to the development of

Table 1
Baseline characteristic of Vietnamese patients treated with or without TDF.

Variables	Without TDF	With TDF	*P* value
Number of patients (%)	403 (72.5)	153 (27.5)	
Age, years	35.6 ± 7.0	36.9 ± 6.8	0.064
Women, *n* (%)	167 (41.4)	45 (29.4)	0.009
Body weight	55.7 ± 8.3	56.5 ± 8.2	0.284
Serum creatinine, mg/dl	0.93 ± 0.13	0.93 ± 0.12	0.668
CD4+ cell count, cell/μl	394 ± 197	385 ± 166	0.651
Log 10 HIV-RNA level, copies/ml	1.48 ± 0.55	1.42 ± 0.41	0.190
Proteinuria, *n* (%)	48 (11.9)	21 (13.7)	0.522
Glucosuria, *n* (%)	3 (0.7)	2 (1.3)	0.617
HBVAg (+), *n* (%)	22 (5.5)	29 (18.9)	<0.001
HCVAb (+), *n* (%)	153 (38.0)	69 (45.1)	0.014
Duration of ART, years	1.14 ± 1.35	1.20 ± 1.47	0.650
Use of ritonavir boosted lopinavir, *n* (%)	7 (1.7)	5 (3.3)	0.326
Use of cotrimoxazole drug, *n* (%)	136 (33.7)	45 (29.4)	0.330
Prior AIDS defining disease, *n* (%)	36 (8.9)	12 (7.8)	0.683
Diabetes mellitus (+), *n* (%)	31 (7.7)	19 (12.4)	0.082

Data are expressed as mean ± SD.
ART = Antiretroviral therapy; TDF = tenofovir.

renal dysfunction by Kaplan–Meier method in the two groups. The incidence of renal dysfunction was significantly higher in the TDF-switched group, compared with the non-TDF group ($p = 0.023$, Log-rank test). With regard to the time of switch to TDF, 109 (71.5%) patients of the TDF-switched group switched their nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) to TDF within 3 months from the baseline and additional 31 (20.0%) switched between 3 and 6 months. Furthermore, of the 19 patients of the TDF-switched group who developed renal dysfunction, 13 (71.2%) switched to TDF within 3 months from the baseline and additional 5 (23.5%) switched to TDF between 3 and 6 months.

Table 2 shows the results of the Cox proportional hazards regression model. Univariate analysis identified body weight per 1 kg-decrement, use of TDF, and glucosuria as factors significantly associated with renal dysfunction. After adjustment by multivariate analysis, body weight per 1 kg-decrement (HR = 1.057; 95%CI, 1.016–1.098; $p = 0.006$), use of TDF (HR = 1.980; 95%CI, 1.094–3.582; $p = 0.024$), and glucosuria (HR = 5.202; 95%CI, 1.245–21.738; $p = 0.024$) were still associated significantly with renal dysfunction.

We also compared the incidence of renal dysfunction in the TDF-switched group according to body weight. Fig. 2 shows the time from baseline to renal dysfunction in patients with body weight of <55 kg, representing the average weight of this study population, and in those with ≥55 kg of the TDF-switched group by Kaplan–Meier method. Patients of the <55 kg group were significantly more likely to develop renal dysfunction [12/66 cases (18.2%), 145.3/1000 person-year] compared to patients of the ≥55 kg group [7/87 cases (8.0%), 57.0/1000 person-year] ($p = 0.040$, Log-rank test).

Table 2
Risk factors for 25% decline in creatinine clearance estimated by uni- and multivariate analyses.

	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Age, per year	1.022	0.984–1.061	0.259			
Women	1.484	0.832–2.646	0.181			
Body weight per 1 kg decrease	1.053	1.013–1.094	0.008	1.057	1.016–1.098	0.006
Serum creatinine >1.1 mg/dl	0.397	0.096–1.636	0.201			
CD4+ cell count per cell/μl	1.001	0.999–1.002	0.227			
HIV-RNA level per log 10 copies/ml	0.887	0.446–1.764	0.733			
Proteinuria	0.474	0.147–1.528	0.211			
Glucosuria	5.372	1.301–22.176	0.020	5.202	1.245–21.738	0.024
HBVAg (+)	1.466	0.622–3.458	0.382			
HCVAb (+)	0.949	0.521–1.728	0.864			
Duration of ART per year	1.151	0.970–1.367	0.108			
Use of tenofovir	1.927	1.071–3.465	0.029	1.980	1.094–3.582	0.024
Use of ritonavir boosted lopinavir	2.024	0.491–8.349	0.329			
Use of cotrimoxazole	0.663	0.337–1.305	0.234			
Prior AIDS defining disease	0.043	0.000–4.144	0.177			
Diabetes mellitus (+)	0.952	0.341–2.654	0.925			

HR = hazard ratio; CI = confidence interval; ART = antiretroviral therapy.

The mean serum creatinine was higher in the TDF-switched group compared with the non-TDF group, and the difference in the mean serum creatinine between the two groups increased from 0 mg/dl at baseline, to 0.4 mg/dl at 6 month, 0.5 mg/dl at 12 months and 0.6 mg/dl at 18 months from the baseline.

4. Discussion

In this 18-month prospective study of a single-center cohort, we evaluated the impact of TDF on renal function in Vietnamese HIV-infected patients with low body weight of approximately 55 kg. The Kaplan–Meier curve showed that the cumulative incidence of renal dysfunction was significantly higher among the patients who switched to TDF than among those who did not ($p = 0.023$). Cox proportional hazards regression model identified the use of TDF, low body weight and glucosuria as significant high risk factors for renal dysfunction. In sub-analysis of the TDF-switched group, we confirmed that the cumulative incidence of renal dysfunction was significantly higher in patients with body weight <55 kg compared to those weighing ≥55 kg.

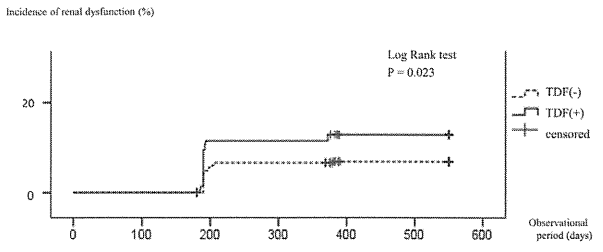

Fig. 1. Kaplan–Meier curve showing the time to renal dysfunction in patients of TDF-switched group and non-TDF-containing groups. Compared to patients of the non-TDF group, those of the TDF-switched group were significantly more likely to develop renal dysfunction ($p = 0.023$, Log-rank test).

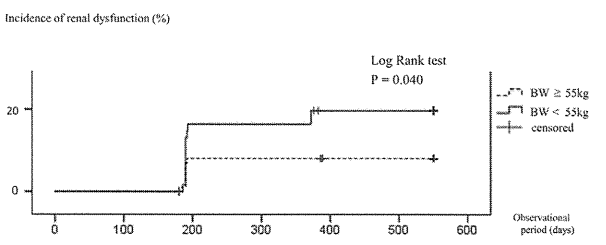

Fig. 2. Kaplan–Meier curve showing the time to renal dysfunction in patients of TDF-switched group classified according to body weight. Compared to patients with body weight ≥55 kg, those weighing <55 kg were significantly likely to develop renal dysfunction ($p = 0.040$, Log-rank test).

We reported previously that low body weight and TDF use were factors significantly associated with chronic kidney disease in a cross-sectional study of this cohort in Hanoi [12]. The present study confirmed that TDF exposure and low body weight bear a causative relationship to renal dysfunction. We also reported low body weight (<59 kg) as a risk factor for renal dysfunction in Japanese patients treated with TDF [10], whereas high body weight of >67 kg was not the risk, similar to the body weight of the patients reported by Cooper et al. [8]. In light of the fact that the average body weight of the patients in this cohort was 55 kg, which is around 30 kg lighter than that of average American males (88 kg) (URL:http://www.cdc.gov/nchs/data/nhsr/nhsr010.pdf), the impact of these risk factors on renal function remain unknown in patients with low body weight in the long-run, thus, observational studies will need to be continued for a longer term.

In addition to low body weight, the presence of glucosuria at baseline was identified as a risk factor for renal dysfunction. This result is consistent with the most recent WHO guidelines which suggest urinary glucose as one of the cost-effective screening test for serious TDF-induced kidney injury (URL: http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf). Since the number of patients with glucosuria was small in this study (about 1% of total population), and glucosuria was not followed until the end of the observation period, further evaluation of this factor is necessary.

Other risk factors for renal dysfunction described in previous studies, such as cotrimoxazole, LPV/r, hepatitis C virus co-infection and diabetes mellitus [13–16] were not identified as risk factors in this study. This discrepancy could be explained by the fact that patients who could be affected by these factors were already excluded according to the study design, which excluded patients with renal dysfunction. With regard to the use of LPV/r, which is known as a risk for renal dysfunction [14,17], especially in cases of co-use with TDF, a number of patients with LPV/r were excluded from the study since most of the patients with LPV/r were co-treated with TDF at baseline. Thus, the impact of co-use of LPV/r and TDF on renal function could be underestimated in this study. Given that LPV/r is used as a salvage regimen and often administered with TDF in Vietnam, long-term monitoring of renal function is required in patients treated with both LPV/r and TDF.

The present study has several limitations. First, data on hypertension, which is a risk factor for renal dysfunction, were not available in this study. Although the average age of patients in this study was around 36 years and the prevalence of hypertension may not high, measurement of blood pressure could lead to better management of renal dysfunction and hypertension should be evaluated for potential risk. Regarding diabetes mellitus as well, the degree of diabetes mellitus was not checked in detail. However, severe patients such as insulin dependence were not in this study, thus, the lack of data could be limited. Second, the observation period of 18 months is relatively short to evaluate long-term adverse event for renal function as mentioned above. Some studies advocated stabilization of decline in eGFR later after the first 6 months of TDF exposure [18] and reversibility of eGFR decline after cessation of TDF therapy [19], while several studies argued incomplete reversibility of eGFR decline following TDF exposure [20–22]. In this study, most of the patients who developed the decline in Crcl continued the same ART regimen because of their moderate and/or stabilized renal dysfunction. However, the observational period of the present study is relatively short compared to other studies, thus, whether or not the stabilization and reversibility will be observed in this cohort of averagely small body weight should be evaluated in the longer period.

Third, the timing of switch to TDF and total duration of ART were not unified in the present study, since the study was an observational cohort in which patients were already on ART at enrollment. The reasons for switch to TDF were mainly related to adverse events caused by d4T and AZT or treatment for HBV infection, thus the timing of switch to TDF was not strictly controlled. However, more than 70% and 90% of the patients were switched to TDF within 3 and 6 months from baseline, respectively, thus influence of this limitation on the result of this study could be restricted.

Despite concern on nephrotoxicity, TDF remains an important drug with enough anti-HIV potency and less mitochondrial toxicity among NRTIs. In order to use it safely in the long term, serum creatinine should be monitored in patients with aforementioned risk factors even in resource-limited situations. Further longitudinal studies are required to determine the impact of TDF, low body weight and glucosuria on renal function in Vietnamese and other Asian people with low body weight.

Conflict of interest

S.O. has received honoraria and research grants from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Pfizer, Co., and Roche Diagnostics K.K.; received honoraria from Astellas Pharmaceutical K.K., Bristol-Myers K.K., Daiichisankyo, Co., Dainippon Sumitomo Pharma, Co., GlaxoSmithKline, K.K., Taisho Toyama Pharmaceutical, Co., Torii Pharmaceutical, Co., and ViiV Healthcare. H.G. has received honoraria from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Torii Pharmaceutical, Co., and ViiV Healthcare, Co. All other authors declare no conflict of interest.

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Selection of TI8-8V Mutant Associated with Long-Term Control of HIV-1 by Cross-Reactive HLA-B*51:01–Restricted Cytotoxic T Cells

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Elite controllers of HIV-1–infected HLA-B*51:01$^+$ hemophiliacs, who remain disease free and have a very low plasma viral load for >30 y, had the 8V mutation at an immunodominant Pol283-8 (TI8) epitope, whereas the 8T mutant was predominantly selected in other HIV-1–infected HLA-B*51:01$^+$ hemophiliacs, suggesting an important role of the 8V mutant selection in long-term control of HIV-1. However, the mechanism of this selection and the long-term control in these elite controllers remains unknown. In this study, we investigated the mechanism of the 8V mutant selection in these controllers. TI8-specific CTLs from these individuals evenly recognized both TI8 peptide–pulsed and TI8-8V peptide–pulsed cells and effectively suppressed replication of wild-type (WT) and the 8V viruses. However, the results of a competitive viral suppression assay demonstrated that CTLs from the individual who had WT virus could discriminate WT virus from the 8V virus, whereas those from the individuals who had the 8V virus evenly recognized both viruses. The former CTLs carried TCRs with weaker affinity for the HLA-B*51:01-TI8-8V molecule than for the HLA-B*51:01-TI-8 one, whereas the latter ones carried TCRs with similar affinity for both molecules. The reconstruction of the TCRs from these CTLs in TCR-deficient cells confirmed the different recognition of the TCRs for these epitopes. The present study showed that the 8V mutant virus could be selected by cross-reactive CTLs carrying TCR that could discriminate a small difference between the two molecules. The selection of the 8V mutant and elicitation of these two cross-reactive CTLs may contribute to the long-term control of HIV-1. *The Journal of Immunology*, **2014, 193: 4814–4822.**

Cytotoxic T lymphocytes play an important role in the control of HIV-1 (1–9). However, HIV-1 can escape from CTL-mediated immune pressure by various mechanisms such as Nef-mediated HLA class I downregulation and mutation to allow escape from HIV-1–specific CTLs (10, 11). The acquisition of amino acid mutations within CTL epitopes and/or its flanking regions leads to reduced ability for peptide binding to HLA class I molecules, impaired TCR recognition, and defective epitope generation (12, 13), resulting in lack of CTL activities to suppress replication of HIV-1 mutant virus as well as in the selection and accumulation of escape mutant viruses (10, 14–19).

A minority of HIV-1–infected individuals, who are known as elite controller or long-term nonprogressors, remain disease free and have a very low viral load (VL), even in the absence of antiretroviral therapy (20–22). A majority of these elite controllers carry the HLA-B*57/58:01, HLA-B*27, or HLA-B*51 allele associated with slow progression to AIDS (23, 24), suggesting that HIV-1–specific CTLs restricted by these HLA alleles control HIV-1 in elite controllers. The mechanism of the control by these CTLs has been well studied in elite controllers and slow progressors carrying HLA-B*57/58:01, HLA-B*27 or HLA-B*13. These studies showed strong Gag-specific CD8$^+$ T cell responses in elite controllers or slow progressors carrying these alleles, suggesting that they may control HIV-1 (15, 16, 25–27). HLA-B*57–mediated immune pressure selects the escape mutation T242N in the Gag TW10 epitope. This mutation impairs viral replication, resulting in control of HIV-1 in these HLA-B*57$^+$ individuals (28, 29). In the case of HLA-B*27$^+$ individuals, the presence of Gag KK10–specific CD8$^+$ T cell is associated with the control of HIV-1 (4, 30–32). The immunodominant KK10 epitope is almost invariably targeted by CD8$^+$ T cells, and the KK10-specific CD8$^+$ T cells display potent effector functions (4, 30, 31, 33). The conservation of this response is thought to account for the control of HIV-1 in these individuals.

A previous study showed that the HLA-B*51:01 allele was associated with long-term control of HIV-1 in HIV-1–infected Japanese hemophiliacs, and the frequency of HLA-B*51:01–restricted Pol283-290 (TI8: TAFTIPSI)–specific CD8$^+$ T cells was inversely associated with plasma VL in HIV-1–infected ones (34), suggesting an important role of TI8-specific CD8$^+$ T cells in the long-term control of HIV-1 infections. Four mutations (8T, 8L, 8R, and 8V) at position 8 of the TI8 epitope were significantly detectable in HLA-B*51$^+$ individuals more than in HLA-B*51$^-$ individuals, suggesting that these mutations were selected by TI8-specific CTLs (35). The 8T mutation is predominantly found in HIV-1–infected HLA-B*51:01$^+$ donors. TI8-specific CTLs have a strong ability to suppress the replication of wild-type (WT) and the 8V mutant viruses in vitro but fail to suppress that of the 8T, 8L, and 8R mutant viruses (35, 36). A study using a Japanese hemophiliac cohort showed that the 8V mutation is found in only

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Abbreviations used in this article: 7-AAD, 7-aminoactinomycin D; RT, reverse transcriptase; VL, viral load; WT, wild-type.

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HLA-B*B51:01$^+$ elite controllers (34), suggesting that the selection of the 8V mutant virus is a critical factor for long-term control of HIV-1 in Japanese hemophiliacs. However, the mechanism underlying selection of the 8V virus in HLA-B*51:01$^+$ elite controllers remains unclear.

In the present study, to clarify the mechanisms of the 8V mutant selection, we investigated how TI8-specific CTLs from elite controllers select the 8V mutant. We established TI8-specific CTL clones from three elite controllers and then analyzed their abilities to suppress the replication of the 8V virus and to select this mutant in vitro. In addition, we assessed the TCR affinity of the CTLs for HLA-B*51:01 with the 8V peptide or WT peptide and evaluated the function of TCRs isolated from the CTL clones by reconstructing them in TCR-deficient cells. In the present study, we clarified the mechanism of the 8V selection and suggested its role in the long-term control of HIV-1 in Japanese hemophiliacs.

Materials and Methods

Patients

Three HIV-1–infected, antiretroviral-naive Japanese hemophiliacs were recruited for the current study, which was approved by the ethics committees of Kumamoto University (RINRI number 540, GENOME number 210) and the National Center for Global Health and Medicine (ID-NCGM-A-000172-00). Written informed consent was obtained from all subjects according to the Declaration of Helsinki.

Cells

C1R cells were purchased from American Type Culture Collection. C1R cells expressing HLA-B*51:01(C1R-B*51:01) were previously generated by transfecting C1R cells with *HLA-A*51:01* genes (37, 38). They were maintained in RPMI 1640 medium containing 5% FBS (R5) and 0.15 mg/ml hygromycin B. TCR-deficient mouse T cell hybridoma cell line TG40 cells were provided by T. Saito (RIKEN Institute, Saitama, Japan). TG40 cells expressing human CD8α (TG40/CD8) were previously established by transfecting TG40 cells with CD8α genes (39). TG40/CD8 and T1 cells, purchased from American Type Culture Collection, were maintained in R5.

HIV-1 clones

A previously reported infectious proviral clone of HIV-1, pNL-432, was used (40). Pol283-8V mutant viruses were previously generated on the basis of pNL-432 (34, 35).

Generation of TI8-specific CTL clones

HLA-B*51:01–restricted TI8-specific CTL clones were generated from HIV-1–specific bulk-cultured T cells by limiting dilution in U-bottom 96-well microtiter plates (Nunc). Each well contained 200 μl cloning mixture (~1×10^6 irradiated allogeneic PBMCs from healthy donors and 1×10^5 irradiated C1R-B*51:01 cells prepulsed with the corresponding peptide at 1 μM in RPMI 1640 medium containing 10% FBS [R10], 200 U/ml human rIL-2, and 2.5% PHA soup). These CTL clones were cultured in RPMI 1640 medium containing 10% FBS, 200 U/ml human rIL-2, and 2.5% PHA soup. The CTL clones were stimulated biweekly with irradiated target cells pulsed with the peptide.

Cytotoxic assay of CTL clones

The cytotoxic activity of TI8-specific CTL clones was determined by the standard ^{51}Cr release assay described previously (36). Briefly, C1R-B*51:01 cells were incubated with 100 μCi $Na_2{}^{51}CrO_4$ in saline for 60 min and then washed three times with R5. Labeled target cells (2×10^3/well) were added to each well of a U-bottom 96-well microtiter plate (Nunc) with the appropriate amount of the corresponding peptide. After a 1-h incubation, effector cells were added at an E:T ratio of 2:1, and then, the cultures were incubated for 4 h at 37°C. The supernatants were collected and analyzed with a gamma counter. Spontaneous ^{51}Cr release was determined by measuring the number of counts per minute in supernatants from wells containing only target cells (cpm spn). Maximum ^{51}Cr release was determined by measuring the cpm in supernatants from wells containing target cells in the presence of 2.5% Triton X-100 (cpm max). Specific lysis was defined as (cpm exp − cpm spn)/(cpm max − cpm spn) × 100, where cpm exp is the number of cpm in the supernatant in the wells containing both target and effector cells.

HIV-1 replication suppression assay

The ability of TI8-specific CTL clones to suppress HIV-1 replication was examined as described previously (41). CD4$^+$ T cells were isolated from PBMCs of HLA-B*51:01$^+$ healthy donors and incubated with the desired HIV-1 clones for 4 h at 37°C. After three washes with R5, the cells were cocultured with TI8-specific CTL clones. From days 3 to 7 post infection, culture supernatants were collected, and the concentration of p24 Ag in them was measured by use of an ELISA kit (HIV-1 p24 Ag ELISA kit; ZeptoMetrix).

In vitro competitive viral suppression assay

T1 cells (HLA-B*51:01$^+$) were coinfected with NL-432 and NL432-Pol283-8V mutant viruses at a ratio of 9:1. The infected cells were incubated with TI8-specific CTL clones at an E:T ratio of 0.05:1. From days 4 to 7 postinfection, culture supernatants were collected; and the concentration of p24 Ag in these supernatants was measured by using the HIV-1 p24 Ag ELISA kit (ZeptoMetrix). Viral RNA was extracted from the culture supernatant by using a QIAamp Viral RNA Mini kit (Qiagen) and subjected to RT-PCR by use of a SuperScript III One-Step RT-PCR System (Invitrogen). Nested PCR was subsequently performed for direct sequencing. The ratio of WT to mutant virus was determined by the relative peak height on the sequencing electrogram.

Tetramer binding assay

HLA class I–peptide tetrameric complexes (tetramers) were generated as described previously (34). CTL clones were stained with PE-conjugated tetramers at 37°C for 30 min. The cells were then washed twice with R5, followed by staining with FITC-conjugated anti-CD8 mAb and 7-amino-actinomycin D (7-AAD) at 4°C for 30 min. Finally, they were washed twice with R5. For the analysis of tetramer association, CTL clones were stained with PE-conjugated tetramer at a concentration of 1 μM at 37°C for various times (0, 2, 5, 10, 15, 30, and 60 min) and then washed as described above. For the analysis of tetramer dissociation, CTL clones were stained with PE-conjugated tetramer at a concentration of 1 μM at 37°C for 30 min. A portion of the cells was then removed periodically (0, 5, 10, 15, 30, 60, 80, and 110 min), and the cells were washed as described above. The cells stained with tetramer were then analyzed by flow cytometry (FACSCanto II).

Sequencing of plasma RNA

Viral RNA was extracted from the plasma of chronically HIV-1–infected individuals by using a QIAamp Mini Elute Virus spin kit (Qiagen). cDNA was synthesized from the RNA with Superscript III and random primer (Invitrogen). We amplified HIV reverse transcriptase (RT) sequences by nested PCR with RT-specific primers 5′-ACACCTGTCAACATAATTGG-3′ and 5′-TGTATGTCATTGACAGTCCA-3′ for the first-round PCR and 5′-GGGCCTGAAAATCCATACAA-3′ and 5′-GGTGATCCTTTCCATCCC-TG-3′ for the second-round PCR. PCR products were sequenced directly or cloned with a TOPO TA cloning kit (Invitrogen) and then sequenced. Sequencing was done with a BigDye Terminator version 1.1. cycle sequencing kit (Applied Biosystems) and analyzed by use of an ABI PRISM 310 or 3100 Genetic Analyzer.

TCR clonotype analysis

CTL clones were stained with PE-conjugated tetramers, anti-CD8 mAb, and 7-AAD, and then, tetramer$^+$CD8$^+$7-AAD$^-$ cells were sorted by using a FACSAria. Unbiased identification of TCR-αβ-chain usage was assessed as described previously (42). TCR gene designations were based on the ImMunoGeneTics database.

Reconstruction of TCRs on TCR-deficient T cells

The cDNAs encoding full-length TCRα and TCRβ of TI8-specific CTL clones were obtained by a previously described method (42). cDNA was then amplified by nested PCR with TCR-α–specific primers 5′-GGAATTCGCCGCCACCATGCTCCTGCTGCTCGTCCCAG-3′ and 5′-ATTTGCGGCCGCAGATCTCAGCTGGACCACAGCCGCAG-3′ or 5′-GGAATTCGCCGCCACCATGGAAACTCTCCTGGGAGTGT-3′ and with TCR-β–specific 5′-GGAATTCGCCGCCACCATGGCCTCCCTGCTCTT-CTTCT-3′ and 5′-ATTTGCGGCCGCCTAGCCTCTGGAATCCTTTCTC-TTGA-3′ or 5′-GGAATTCGCCGCCACCATGGGCACCAGGCTCCTCT-GCT-3′. The amplified genes were separately cloned into a retrovirus vector pMX and used to transfect TG40/CD8, as described previously (39). Briefly, the genes of TCR were subcloned into the retroviral vector pMX. First, the ecotropic virus packaging cell line Platinum-E was transfected with the constructs. Two days later, the culture supernatant containing recombinant

virus was collected and then incubated with TG40/CD8 cells in the presence of 10 μg/ml polybrene for 6 h. The cells were cultured for an additional 2 d for analysis of TCR gene expression. Finally, the cells showing bright staining with PE-conjugated anti-mouse $CD3^+$ mAb (2C11; BD Pharmingen) were sorted by using the FACSAria.

IL-2 secretion assays

TG40/CD8 cells transfected with TCRs (4×10^4/well) were cultured with C1R-B*51:01 (4×10^4/well) in 200 μl R5 in the presence of various concentrations of peptides in U-bottom 96-well microtiter plates (Nunc) for 48 h at 37°C. The culture supernatants were then collected, and the concentration of IL-2 in them was measured by use of an IL-2 ELISA kit (eBioscience).

Results

*Slow selection of Pol283-8V mutant in HLA-B*51:01$^+$ Japanese elite controllers*

We previously revealed that only 3 (patients KI-021, KI-051, and KI-124) of 108 Japanese hemophiliacs who had survived without antiretroviral therapy for ~15 y, from 1983 to 1998, exhibited a strong inhibition of HIV-1 for an additional 10 y (34). All three of these elite controllers recruited from 1997 to 1999 had HLA-B*51:01 and the 8V mutation at position 8 in the TI8 epitope. To identify when the 8V emerged, we performed longitudinal sequence analysis of the TI8 epitope in these three patients. KI-021 and KI-124 already had the 8V mutant in September 1997 and January 1998, respectively. KI-051 had only the WT sequence in October 1999, the 8V mutant in 63% of the clones analyzed in July 2002, and then only the 8V mutant in October 2006 (Table I). Since KI-051 had been infected with HIV-1 before 1985, these results indicate that the WT virus predominantly existed for >17 y after HIV-1 infection. Thus, this mutant was slowly selected and had accumulated in this patient.

Recognition of the 8V mutant by TI8-specific CTLs

A previous study showed that the 8V mutation weakly reduced the recognition of TI8-specific CTL clones established from KI-051 in July 2002 (34, 35). We first reconfirmed this finding by using three TI8-specific CTL clones (2B5, 2C6, and 2D1), which were established from KI-051 in July 2002 when the WT virus was still detectable. The results for a representative CTL clone (2C6 clone) are shown in Fig. 1A. This clone effectively suppressed both WT and 8V mutant viruses but revealed slightly weaker ability to suppress the replication of the 8V mutant than the WT virus at higher E:T ratios. All three CTL clones revealed significantly weaker ability to suppress the replication of the 8V mutant than the WT virus at an E:T cell ratio of 1:1 (Fig. 1B, 1C). We further established 11 TI8-specific CTL clones from three elite controllers (KI-051 in June 2009, KI-021 in September 1997 and January 2005, and KI-124 in August 2001) and investigated the ability of these TI8-specific CTL clones to recognize the WT and the mutant epitopes. We first measured the killing activity toward target cells prepulsed with the WT or the 8V mutant peptide. All 11 CTL clones as well as 2B5, 2C6, and 2D1 clones showed the same killing activity toward target cells prepulsed with the 8V peptide as that of those prepulsed with the WT peptide (Supplemental Fig. 1). We next analyzed the ability of these CTL clones to suppress the replication of the 8V virus. CTL clones 2B, 7B, and 7F, which were established from KI-051 in June 2009 when only the 8V virus was detectable, exhibited a similar ability to suppress both the 8V and the WT virus at the same level (Fig. 1B, 1C). The CTL clones from KI-021 and KI-124 also showed characteristics similar to those of these clones from KI-051 (Fig. 1B, 1C).

Table I. Sequence of Pol283-8 epitope in three HLA-B*51:01$^+$ elite controllers of HIV-1–infected Japanese hemophiliacs

Patient	HLA Allele				Sample Date (month/date/year)	Pol283-8 Epitope			VL (copies/ml)	CD4 (cells/ml)	Name of CTL Clone
	A Allele		B Allele	C Allele		Sequence	Frequency				
						TAFTIPSI[a]	Direct (%)[b]	Cloning[c]			
KI-021	2402	2602	5101	0702 1402	9/12/1997	- - - - - - - V	100	12/12	<400	727	3B, 4C, 3D
					3/27/1998	- - - - - - - V	100	12/12	<400	808	
					1/27/2005	- - - - - - - V	100	12/12	<50	646	10, 20, 52
KI-051	0206	3101	4002	5101 1402 1502	10/26/1999	- - - - - - - -	100	NT[d]	<400	629	
					7/25/2002	- - - - - - - -	37	6/12	63	911	2B5, 2C6, 2D1
						- - - - - - - V	63	6/12			
					10/24/2006	- - - - - - - V	75	NT[d]	<50	966	
						- - - V - - - V	25	NT[d]			
					6/30/2009	- - - - - - - V	90	9/12	<50	1040	2B, 7B, 7F
						- - - - V - - V	10	3/12			
KI-124	1101	0206	5101	1501 0401 1402	1/13/1998	- - - - - - - V	100	15/15	<400	745	
					8/16/2001	- - - - - - - V	100	18/18	600	511	12E, 12H

[a]The sequences for KI-021 in March 1998 and January 2005 were obtained from proviral DNA, whereas those for all other patients and KI-021 in September 1997 were from plasma RNA.
[b]Direct, direct sequence.
[c]Number of clones carrying the indicated sequence/number of clones tested.
[d]NT, not tested.