Table 6. Number of patients whose eGFR decreased to <60 ml/min/1.73 m^2 and who discontinued tenofovir with clinical diagnosis of renal dysfunction due to tenofovir.

	<59 kg (n = 167)	59–67 kg (n = 168)	>67 kg (n = 160)	p value
eGFR <60 ml/min/1.73 m^2	4 (2.4%)	1 (0.6%)	1 (0.6%)	0.229
Discontinued tenofovir	16 (9.6%)	8 (4.8%)	1 (0.6%)	0.001
Reasons for discontinuation				
>25% eGFR decrement	8 (4.8%)	4 (2.4%)	0 (0%)	
Urine β2 microglobulin >5000 μg/l	11 (6.6%)	4 (2.4%)	1 (0.6%)	

Among the patients who discontinued tenofovir, both >25% fall in eGFR and urine β2 microglobulin >5000 μg/l were registered in six patients with body weight <59 kg, and in three patients with body weight 59–67 kg.
eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0022661.t006

(Table 3, Model 3 and Table 4, Model 3). However, these results have to be interpreted with caution, because these multivariate analyses were formulated to primarily evaluate weight decrement, not age or smoking.

The mechanism of TDF-associated renal dysfunction is not fully understood. TDF-associated renal dysfunction probably develops as a result of complex interaction of pharmacological, environmental, and genetic factors, rather than small body weight only [32]. It should be noted, however, that small body weight has been identified as a risk factor for TDF-associated renal dysfunction not only in clinical trials, but also in *in vitro* and pharmacokinetic studies [33–36]. TDF is the prodrug of acyclic nucleotide analog tenofovir, which is excreted by both glomerular filtration and active tubular secretion. *In vitro* studies showed that tenofovir exhibits mitochondrial toxicity in renal proximal tubular cells, and animal studies demonstrated that renal tubular dysfunction was associated with the dose and plasma drug concentrations of TDF [34,35]. Furthermore, pharmacokinetic studies showed that small body weight is associated with reduced plasma TDF clearance and thus high plasma TDF concentrations, which could result in renal tubular dysfunction. [33,36].

There are several limitations to our study. First, because of the retrospective nature of the study, patients with possible risks for TDF-associated renal dysfunction could have not been prescribed TDF. Because of this selection bias, the incidence of TDF-associated renal dysfunction might be underestimated. Second, the study did not compare the incidence of renal dysfunction in a control group (TDF-free ART). Due to the small body weight in Japanese or other factors such as genetics, the use of ART without TDF might cause higher incidence of renal dysfunction as well. Third, as discussed above, the definition of TDF-associated renal dysfunction, especially the criteria used to evaluate proximal renal tubular damage, is not uniformly established in the field and is different in the published studies. Accordingly, we decided to adopt changes in eGFR, instead of parameters for proximal renal

tubular damage. Using the eGFR as a marker for TDF-associated renal dysfunction, our results might have underestimated the incidence of TDF-associated renal dysfunction.

In conclusion, the present study demonstrated a high incidence of TDF-associated renal dysfunction among Japanese patients, a potentially high-risk group due to the low median body weight. The results also identified small body weight as a risk for TDF-associated renal dysfunction in a statistical model that included small body weight as a primary exposure. TDF is certainly a drug of choice for one of the components of the first line therapies for HIV infection. However, the importance of close monitoring for renal function in patients with small body weight should be emphasized for early detection of TDF-associated renal dysfunction.

Supporting Information

Text S1 Letter of Approval from Human Research Ethics Committee of National Center for Global Health and Medicine.
(PDF)

Dataset S1 Raw data of the target population.
(XLS)

Author Contributions

Conceived and designed the experiments: AN TH KU TN MKS. Performed the experiments: AN H. Sakai. Analyzed the data: AN TH KU KO IK H. Sakai. TN MKS. Contributed reagents/materials/analysis tools: H. Suemori H. Sakai. Wrote the manuscript: AN TN MKS.

References

1. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, et al. (2006) Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med 354: 251–260.
2. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, et al. (2004) Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA 292: 191–201.
3. Izzedine H, Hulot JS, Vittecoq D, Gallant JE, Staszewski S, et al. (2005) Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naive HIV-1-infected patients. Data from a double-blind randomized active-controlled multicentre study. Nephrol Dial Transplant 20: 743–746.

4. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, et al. (2007) The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. AIDS 21: 1273–1281.
5. Arribas JR, Pozniak AL, Gallant JE, Dejesus E, Gazzard B, et al. (2008) Tenofovir disoproxil fumarate, emtricitabine, and efavirenz compared with zidovudine/lamivudine and efavirenz in treatment-naive patients: 144-week analysis. J Acquir Immune Defic Syndr 47: 74–78.
6. Post FA, Moyle GJ, Stellbrink HJ, Domingo P, Podzamczer D, et al. (2010) Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavir-

120

enz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. J Acquir Immune Defic Syndr 55: 49–57.

7. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, et al. (2002) Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney Dis 40: 1331–1333.

8. Schaaf B, Aries SP, Kramme E, Steinhoff J, Dalhoff K (2003) Acute renal failure associated with tenofovir treatment in a patient with acquired immunodeficiency syndrome. Clin Infect Dis 37: e41–43.

9. Rollot F, Nazal EM, Chauvelot-Moachon L, Kelaidi C, Daniel N, et al. (2003) Tenofovir-related Fanconi syndrome with nephrogenic diabetes insipidus in a patient with acquired immunodeficiency syndrome: the role of lopinavir-ritonavir-didanosine. Clin Infect Dis 37: e174–176.

10. Peyriere H, Reynes J, Rouanet I, Daniel N, de Boever CM, et al. (2004) Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. J Acquir Immune Defic Syndr 35: 269–273.

11. Kinai E, Hanabusa H (2009) Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. AIDS Res Hum Retroviruses 25: 387–394.

12. Winston A, Amin J, Mallon P, Marriott D, Carr A, et al. (2006) Minor changes in calculated creatinine clearance and anion-gap are associated with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy. HIV Med 7: 105–111.

13. Gallant JE, Winston JA, DeJesus E, Pozniak AL, Chen SS, et al. (2008) The 3-year renal safety of a tenofovir disoproxil fumarate vs. a thymidine analogue-containing regimen in antiretroviral-naive patients. AIDS 22: 2155–2163.

14. Fux CA, Simcock M, Wolbers M, Bucher HC, Hirschel B, et al. (2007) Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study. Antivir Ther 12: 1165–1173.

15. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, et al. (2010) Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis 51: 496–505.

16. Gayet-Ageron A, Ananworanich J, Jupimai T, Chetchotisakd P, Prasithsirikul W, et al. (2007) No change in calculated creatinine clearance after tenofovir initiation among Thai patients. J Antimicrob Chemother 59: 1034–1037.

17. Chaisiri K, Bowonwatanuwong C, Kasettratat N, Kiertiburanakul S (2010) Incidence and risk factors for tenofovir-associated renal function decline among Thai HIV-infected patients with low-body weight. Curr HIV Res 8: 504–509.

18. Reid A, Stohr W, Walker AS, Williams IG, Kityo C, et al. (2008) Severe renal dysfunction and risk factors associated with renal impairment in HIV-infected adults in Africa initiating antiretroviral therapy. Clin Infect Dis 46: 1271–1281.

19. Gatanaga H, Tachikawa N, Kikuchi Y, Teruya K, Genka I, et al. (2006) Urinary beta2-microglobulin as a possible sensitive marker for renal injury caused by tenofovir disoproxil fumarate. AIDS Res Hum Retroviruses 22: 744–748.

20. Goicoechea M, Liu S, Best B, Sun S, Jain S, et al. (2008) Greater tenofovir-associated renal function decline with protease inhibitor-based versus nonnucleoside reverse-transcriptase inhibitor-based therapy. J Infect Dis 197: 102–108.

21. Rodriguez-Novoa S, Labarga P, Soriano V, Egan D, Albalater M, et al. (2009) Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. Clin Infect Dis 48: e108–116.

22. Bash LD, Coresh J, Kottgen A, Parekh RS, Fulop T, et al. (2009) Defining incident chronic kidney disease in the research setting: The ARIC Study. Am J Epidemiol 170: 414–424.

23. Chue CD, Edwards NC, Davis LJ, Steeds RP, Townend JN, et al. (2011) Serum phosphate but not pulse wave velocity predicts decline in renal function in patients with early chronic kidney disease. Nephrol Dial Transplant;doi: 10.1093/ndt/gfq78.

24. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, et al. (2006) Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med 145: 247–254.

25. Gupta SK, Eustace JA, Winston JA, Boydstun II, Ahuja TS, et al. (2005) Guidelines for the management of chronic kidney disease in HIV-infected patients: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis 40: 1559–1585.

26. Lomaestro BM (2000) Fluoroquinolone-induced renal failure. Drug Saf 22: 479–485.

27. Wever K, van Agtmael MA, Carr A (2010) Incomplete reversibility of tenofovir-related renal toxicity in HIV-infected men. J Acquir Immune Defic Syndr 55: 78–81.

28. Szczech LA, Hoover DR, Feldman JG, Cohen MH, Gange SJ, et al. (2004) Association between renal disease and outcomes among HIV-infected women receiving or not receiving antiretroviral therapy. Clin Infect Dis 39: 1199–1206.

29. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, et al. (2008) Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clin J Am Soc Nephrol 3: 348–354.

30. Antoniou T, Raboud J, Chirhin S, Yoong D, Govan V, et al. (2005) Incidence of and risk factors for tenofovir-induced nephrotoxicity: a retrospective cohort study. HIV Med 6: 284–290.

31. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, et al. (2010) Impact of tenofovir on renal function in HIV-infected, antiretroviral-naive patients. J Acquir Immune Defic Syndr 53: 62–69.

32. Post FA, Holt SG (2009) Recent developments in HIV and the kidney. Curr Opin Infect Dis 22: 43–48.

33. Jullien V, Treluyer JM, Rey E, Jaffray P, Krivine A, et al. (2005) Population pharmacokinetics of tenofovir in human immunodeficiency virus-infected patients taking highly active antiretroviral therapy. Antimicrob Agents Chemother 49: 3361–3366.

34. Van Rompay KK, Durand-Gasselin L, Brignolo LL, Ray AS, Abel K, et al. (2008) Chronic administration of tenofovir to rhesus macaques from infancy through adulthood and pregnancy: summary of pharmacokinetics and biological and virological effects. Antimicrob Agents Chemother 52: 3144–3160.

35. Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, Johnson DM, et al. (2009) Tenofovir renal toxicity targets mitochondria of renal proximal tubules. Lab Invest 89: 513–519.

36. Kiser JJ, Fletcher CV, Flynn PM, Cunningham CK, Wilson CM, et al. (2008) Pharmacokinetics of antiretroviral regimens containing tenofovir disoproxil fumarate and atazanavir-ritonavir in adolescents and young adults with human immunodeficiency virus infection. Antimicrob Agents Chemother 52: 631–637.

121

Amebiasis in HIV-1-Infected Japanese Men: Clinical Features and Response to Therapy

Koji Watanabe[1,2], Hiroyuki Gatanaga[1,2]*, Aleyla Escueta-de Cadiz[3], Junko Tanuma[1], Tomoyoshi Nozaki[3], Shinichi Oka[1,2]

1 AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, 2 Center for AIDS Research, Kumamoto University, Kumamoto, Japan, 3 Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan

Abstract

Invasive amebic diseases caused by *Entamoeba histolytica* are increasing among men who have sex with men and co-infection of ameba and HIV-1 is an emerging problem in developed East Asian countries. To characterize the clinical and epidemiological features of invasive amebiasis in HIV-1 patients, the medical records of 170 co-infected cases were analyzed retrospectively, and *E. histolytica* genotype was assayed in 14 cases. In this series of HIV-1-infected patients, clinical presentation of invasive amebiasis was similar to that described in the normal host. High fever, leukocytosis and high CRP were associated with extraluminal amebic diseases. Two cases died from amebic colitis (resulting in intestinal perforation in one and gastrointestinal bleeding in one), and three cases died from causes unrelated to amebiasis. Treatment with metronidazole or tinidazole was successful in the other 165 cases. Luminal treatment was provided to 83 patients following metronidazole or tinidazole treatment. However, amebiasis recurred in 6 of these, a frequency similar to that seen in patients who did not receive luminal treatment. Recurrence was more frequent in HCV-antibody positive individuals and those who acquired syphilis during the follow-up period. Various genotypes of *E. histolytica* were identified in 14 patients but there was no correlation between genotype and clinical features. The outcome of metronidazole and tinidazole treatment of uncomplicated amebiasis was excellent even in HIV-1-infected individuals. Luminal treatment following metronidazole or tinidazole treatment does not reduce recurrence of amebiasis in high risk populations probably due to amebic re-infection.

Introduction

Invasive amebiasis (IA) caused by *Entamoeba histolytica* is the second most common cause of mortality associated with parasitic infections worldwide, accounting for 40,000 to 100,000 deaths annually [1]. Amebiasis is transmitted by ingestion of food or water containing the cyst form of *E. histolytica*, which is prevalent in developing countries in Central and South America, Asia, and Africa. In the developed countries, most cases arise in travelers and immigrants from such endemic areas [2]. Recently, however, three developed East Asian countries (Japan, Taiwan, and South Korea) reported increased risk for amebiasis among men who have sex with men (MSM) due to oral-anal sexual contact [3–12]. The annual incidence of human immunodeficiency virus type 1 (HIV-1) infection is also increasing among MSM in these countries [13–17], resulting in growing concern on IA in HIV-1-infected MSM [6,9–12,18]. The recommended treatment for IA is metronidazole (750 mg t. i. d. for 10 days) or tinidazole (2 g q. d. for 3 days), followed by a luminal agent (paromomycin 500 mg t. i. d. for 10 days or diloxanide furoate 500 mg t. i. d. for 10 days) to eliminate intestinal colonization [18,19]. A previous report described no difference in the response to metronidazole or tinidazole treatment between HIV-1-positive and –negative IA patients [20]. However, the efficacy of luminal treatment in preventing recurrence, which

can arise by relapse or re-infection, has not yet been assessed rigorously. In this study, we retrospectively analyzed 170 HIV-1-infected Japanese patients with IA, together with genomic typing of *E. histolytica* in 14 of these patients, and delineated the clinical features of IA in HIV-1-infected individuals and the efficacy of metronidazole, tinidazole and luminal treatment.

Methods

Ethics statement

The Institutional Review Board of National Center for Global Health and Medicine (Tokyo, Japan) approved this study. All patients who provided clinical samples for genotyping of *E. histolytica* gave written informed consent.

Case review

The medical records of HIV-1-infected cases diagnosed with IA at the AIDS Clinical Center, National Center for Global Health and Medicine, between April 1997 and March 2010, were reviewed. The diagnosis of IA was made when one of the following criteria was satisfied; 1) identification of and/or positive PCR (methods; see below) in clinical specimens (stool or punctuate-exudate) for erythrophagocytic trophozoites in patients with IA-

Author Summary

Amebiasis is usually transmitted by ingestion of contaminated food or water in developing countries. Recently, however, increased risk for amebiasis among men who have sex with men (MSM) due to oral-anal sexual contact was reported in developed countries, resulting in growing concern on amebiasis in HIV-1-infected MSM. The recommended treatment of amebiasis is metronidazole or tinidazole, followed by a luminal agent to eliminate intestinal cyst colonization. However, the efficacy of luminal treatment in preventing recurrence has not been assessed yet. In this study, we analyzed the medical records of 170 patients with amebiasis and HIV-1 co-infection. Treatment with metronidazole or tinidazole was excellent whereas luminal treatment did not reduce the frequency of recurrence of amebiasis. Recurrence was more frequent in those MSM with signs of sexual activity such as syphilis infection. Luminal treatment following metronidazole or tinidazole treatment does not reduce recurrence of amebiasis in high risk populations.

related symptoms, e.g., fever and liver abscess, or tenesmus and diarrhea, 2) high serum titer (>1:100) for antibody against *E. histolytica* in patients with IA-related symptoms in whom microbiological cultures or histological examination of clinical specimens did not identify any pathogen, and who showed improvement of IA symptoms following metronidazole or tinidazole monotherapy [10–12]. The medical records were surveyed for patients' characteristics, presenting forms of clinical IA [e.g., colitis, amebic liver abscess (ALA), and perianal abscess], HIV-1-induced immunocompromised status, and symptoms, laboratory data and serological markers of other sexually-transmitted diseases (STD) including syphilis, hepatitis B and C viruses (HBV and HCV). After completion of treatment for IA, the medical records were followed-up until March 2010, excluding those cases found to have died or lost to follow-up.

Genotyping of *E. histolytica*

To determine the strains of *E. histolytica* among HIV-1-infected Japanese patients, genotyping of *E. histolytica* was performed in patients who were PCR positive. The PCR method was used for the first time in our clinic for the diagnosis of amebiasis in December 2008, and since then 14 patients had been diagnosed as IA based on a positive PCR. For the PCR, DNAs were extracted from various biological specimens (e.g., stool, colon wash and punctuate-exudate) by using QIAamp DNA stool Mini Kit (Qiagen, Valencia, CA). Polymerase chain reactions were performed with specific sets of primers designed to target each of 6 loci (D-A, S-Q, R-R, A-L, S^{TGA}-D, and N-K) of tRNA-linked polymorphic short tandem repeats (STR), as described previously [21]. The PCR product was sequenced by ABI 3130XL Genetic Analyzer (Applied Biosystem, Foster city, CA) in both forward and reverse directions. Phylogenetic analysis and genotyping were performed as described previously [22].

Statistical analysis

Differences in patients' characteristics and clinical features were examined using the chi-square test or nonparametric test. The cumulative risk for recurrence was analyzed by the Kaplan-Meier method, and differences were tested by the log-rank test. The Cox proportional hazards model was used to assess the impact of luminal treatment on the recurrence rate after adjustment for other factors. The hazard ratio and 95% confidence interval were calculated. P values less than 0.05 were considered to denote statistical

significance. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL).

Results

Clinical data and response to treatment

IA was diagnosed in 170 HIV-1-infected cases between April 1997 and March 2010 (including amebic colitis, n = 102; ALA, n = 63; and perianal abscess, n = 5, Table 1). Thirty-three patients had two of the above three clinical forms of IA. All patients were males and 164/170 (96.5%) were MSM. High rates of positive TPHA (*Treponema pallidum* hemagglutination assay) (71.2%) and HBV exposure (HBs antigen-positive, HBs antibody-positive, or HBc antibody-positive) (60.0%) were observed. No significant differences were seen in CD4 counts, HIV-1 loads, coexisting AIDS definite disease and the proportion of patients treated with antiretrovirals, suggesting that HIV-induced immunocompromised status did not have an impact on the clinical presentation of amebic infection, in agreement with previous data [12]. In cases of amebic colitis (n = 102), diarrhea (69.7%) was the most common symptom followed by dysentery (55.9%) (Table 2). Fever (>37.5°C) was seen in only 20 patients (19.6%), including 5 cases with perforative peritonitis. In cases with ALA (n = 63), fever (95.2%) was the most common symptom followed by abdominal pain (55.6%). Diarrhea (46.0%) and dysentery (19.0%) were only seen in less than half of ALA cases. Single abscess (72.6%) was identified in most cases. Liver abscesses were seen more frequently in the right lobe (70.5%) than the left (9.8%). Nine patients (14.3%) had pleuritis (considered a co-existing disease), as well as abscesses in the right lobe, and 7 of these presented chest pain. Comparison of physical and laboratory data showed higher peak body temperature (BT), leukocyte count and C reactive protein (CRP) in ALA cases (Table 2) and perforative peritonitis cases (data not shown) compared with colitis cases, indicating that high fever, leukocytosis and high CRP could be the signs of extraluminal amebiasis. It is reported that high fever and leukocytosis are also common in ALA patients free of HIV-1 infection, though both parameters were unusually associated with simple amebic colitis [23]. In ALA cases, however, leukocyte count correlated positively with CD4 count (data not shown in tables: Pearson product-moment correlation coefficient 0.36, p value 0.004) and negatively with HIV-RNA load (Pearson product-moment correlation coefficient -0.28, p value 0.03), but CRP correlated neither with CD4 count nor HIV-RNA load (CRP-CD4, $p = 0.81$, CRP-HIV-RNA, $p = 0.32$). There were also no correlations between CD4 count, HIV-RNA load, BT, leukocyte count or CRP and abscess size or number.

All patients were treated with metronidazole (750 mg t. i. d. for 10 days) for IA, with the exception of two who were treated with tinidazole (2 g q. d. for 3 days). Complete remission of all IA symptoms was observed in 165 patients including the two treated with tinidazole. Five cases died within six months after diagnosis of IA; two from complications related to amebic colitis (one peritoneal perforation and one gastrointestinal bleeding), one from malignant lymphoma, one from *Pneumocystis jirovecii* pneumonia, and one from pulmonary thrombosis. The overall mortality rate was 3% in this study, which was comparable to those reported in non-HIV cases [2,23].

Recurrence after treatment

Luminal agents; paromomycin and diloxanide, are not approved in Japan, and they were not always available in our facility during the study period. After completion of IA treatment with metronidazole or tinidazole, luminal agents were administered when available. Consequently, 83 cases were treated with luminal

Table 1. Patient demographics, state of HIV, and serological markers.

	Colitis (n = 102)[1]	ALA (n = 63)[2]	Perianal abscess (n = 5)[3]	All (n = 170)	P value[4]
Age (years) [IQR]	38 [32–43]	37 [31–44]	45	38 [31–44]	0.58
Male sex (%)	102 (100)	63 (100)	5 (100)	170 (100)	–
Homosexual (%)	96 (94.1)	63 (100)	5 (100)	164 (96.5)	0.053
Past History of amebiasis (%)	16 (15.7)	9 (14.3)	1 (20.0)	26 (15.3)	0.81
CD4 count (/μl)	262 [98–398]	271 [123–411]	58	269 [107–403]	0.84
HIV-RNA (log copies/ml)	4.60 [3.89–5.32]	4.66 [3.91–5.11]	5.04	4.66 [3.93–5.28]	0.70
AIDS (%)	18 (17.6)	8 (12.7)	2 (40.0)	28 (16.5)	0.40
ART initiated (%)	18 (17.6)	11 (17.5)	1 (20.0)	30 (17.6)	0.98
TPHA test positive (%)	77 (75.5)	40 (63.5)	4 (80.0)	121 (71.2)	0.10
HBV exposure (%)	59 (57.8)	41 (65.1)	2 (40.0)	102 (60.0)	0.36
HCV Antibody positive (%)	3 (2.9)	3 (4.8)	0 (0)	6 (3.5)	0.42

Data are median [interquartile range: IQR] or number (percentage) of patients.
[1]5 cases of perforative peritonitis are included as co-existing diseases. Four cases were diagnosed coincidentally by colonoscopy in asymptomatic patients.
[2]31 cases of colitis, 1 case of perianal abscess, 9 cases of pleuritis, and 2 cases of peritonitis are included as co-existing diseases.
[3]1 case of colitis is included as co-existing diseases.
[4]Chi-square test or non-parametric test was performed for data of colitis and ALA.
UD: undetectable, ART: anti-retroviral therapy, TPHA test: *Treponema pallidum* Hemagglutination Assay test, HBV exposure: HBsAg-positive or HBsAb-positive, and/or HBc-Ab positive.
doi:10.1371/journal.pntd.0001318.t001

agents; 38 cases with promomycin (500 mg t. i. d. for 10 days) and 45 cases with diloxanide furoate (500 mg t. i. d. for 10 days). No significant differences were seen in patients' characteristics,

Table 2. Clinical features of amoebic colitis and ALA.

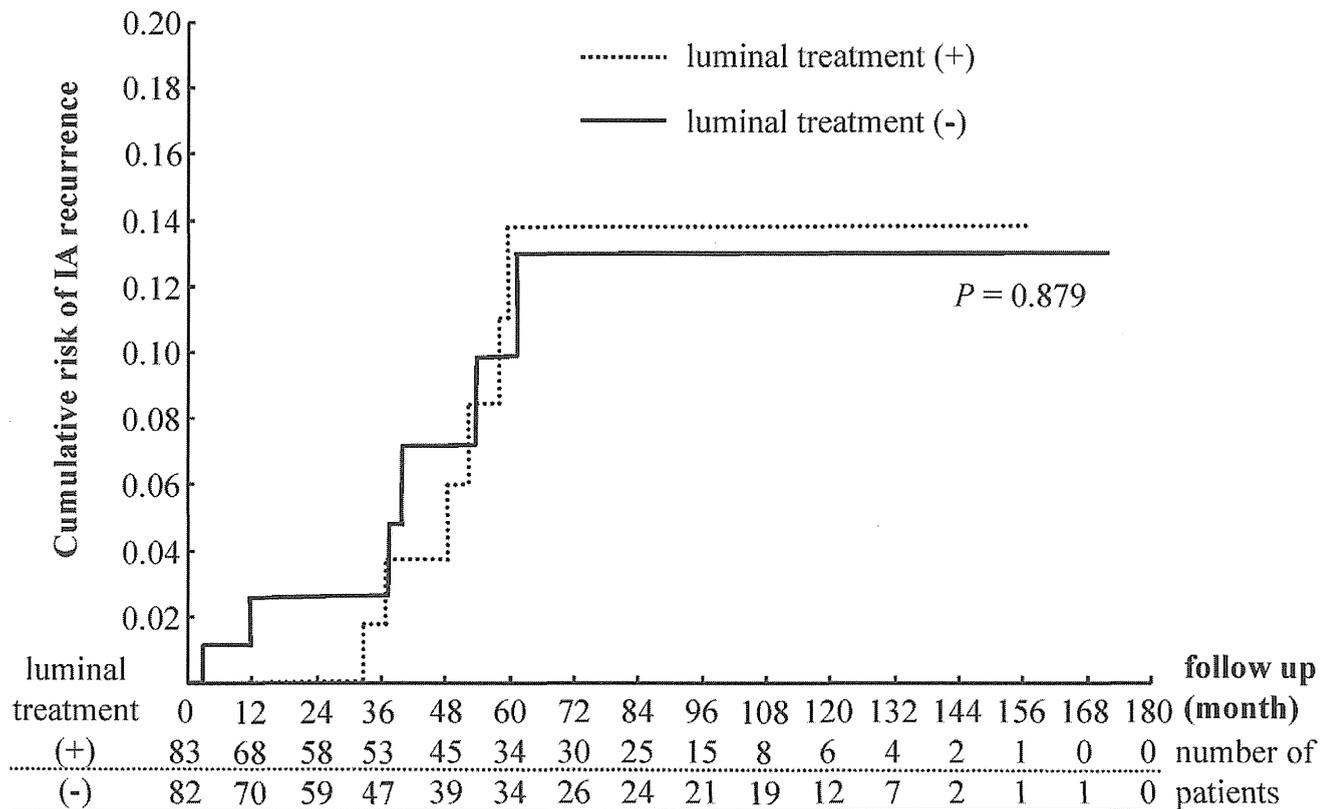|	Colitis (n = 102)	ALA (n = 63)	P value
Symptoms			
Diarrhea (%)	71/102 (69.6)	29/63 (46.0)	0.003
Dysentery (%)	57/102 (55.9)	12/63 (19.0)	<0.001
Abdominal pain (%)	23/102 (22.5)	35/63 (55.6)	<0.001
Chest pain (%)	0/102 (0.0)	7/63 (11.1)	<0.001
Peak BT (°C) [IQR][3]	36.8 [36.5–37.4]	39.0 [38.8–39.5]	<0.001
WBC (/μl) [IQR][3]	5,830 [4490–7580]	11,760 [9460–15170]	<0.001
CRP (mg/dl) [IQR][3]	0.62 [0.16–3.02]	19.15 [10.53–24.75]	<0.001
Frequency of diarrhea[1]			
≤ 5 times/day (%)	63/101 (62.4)	–	
6–10 times (%)	26/101 (25.7)	–	
≥ 11 times (%)	12/101 (11.9)	–	
Size of abscess (mm)	–	59 (10–180)	
Location of abscess[2]			
Right lobe only	–	43/61 (70.5)	
Left lobe only	–	6/61 (9.8)	
Both lobes	–	12/61 (19.7)	
Number of abscesses[1]			
Single (%)	–	45/62 (72.6)	
Multiple (%)	–	17/62 (27.4)	

[1]Data of one case were not available.
[2]Data of two cases were not available.
[3]Data are median [interquartile range: IQR] or number (percentage) of patients.
BT: body temperature, WBC: White Blood Cell counts, CRP: C reactive protein.
doi:10.1371/journal.pntd.0001318.t002

including HIV-1-induced immunocompromised status, serological markers of other STD, and clinical forms and severity of amebiasis between the 83 cases with luminal treatment and 82 cases who did not receive such treatment (Table S1). The median follow-up period after completion of metronidazole or tinidazole treatment was 50 months (inter quartile range: 19–85) in those who received luminal treatment, and 43 months (inter quartile range: 23–98) in those without.

Within the 12-month post-metronidazole treatment period, recurrence of IA was noted in only two patients who did not receive luminal treatment, suggesting reactivation of residual cysts of *E. histolytica* (Figure 1). However, during the entire follow-up period, six in each group experienced recurrence of IA, with no significant difference in the recurrence frequency by the log-rank chi-square test. Multivariate analysis showed that recurrence did not correlate with past history of IA, CD4 count, TPHA, HBV exposure (HBs antigen-positive or HBs antibody-positive), or the presence of extraluminal IA disease (Table 3). However, a positive HCV antibody was significantly associated with IA recurrence. Recurrence also tended to occur in those who acquired new syphilis infection during the follow-up period, though the difference did not reach statistical significance.

Genotypes of *E. histolytica*

Genotyping of *E. histolytica* was performed in samples obtained from 14 patients between December 2009 and March 2010 (colitis, n = 8; ALA, n = 4; colitis and ALA, n = 1; and perianal abscess, n = 1; Table S2). Eleven different genotypes were recognized, including five genotypes (J8, J12, J13, J20, and J23) identified previously in Japan [22], and six newly recognized genotypes (J24-J29). There was no significant relation between *E. histolytica* genotype and clinical presentation.

Discussion

In the present study, retrospective analysis of the medical records of 170 patients with HIV-1-infection and IA showed no

124

Figure 1. Kaplan-Meier estimates of time to IA recurrence. Cumulative probability of IA recurrence after completion of metronidazole or tinidazole treatment with or without subsequent luminal treatment.
doi:10.1371/journal.pntd.0001318.g001

impact for HIV-1-induced immunocompromised status on the clinical forms of amebiasis. The physical and laboratory findings showed that high fever, leukocytosis and high CRP correlated with extraluminal diseases of amebiasis. In ALA cases, however, leukocyte count correlated positively with CD4 count and negatively with HIV-RNA load, indicating that CRP is more sensitive marker for the detection of the extraluminal diseases in advanced immunocompromised patients.

Only five patients died after the diagnosis of IA; two from IA complications and three from other causes. The results indicate

excellent outcome for HIV-1-infected individuals with uncomplicated amebiasis treated with metronidazole or tinidazole, in agreement with previous reports on HIV and non-HIV cases [2,11,12,20,23]. Based on conventional wisdom and written opinion, adequate management of IA should include treatment with a luminal agent following metronidazole or tinidazole treatment, in order to eradicate residual cysts of *E. histolytica* due to the high rate (40–60%) of luminal colonization [2,23–27]. On the other hand, the results of longitudinal observational studies indicated that asymptomatic cyst carriers rarely develop IA, and

Table 3. Multivariate analyses for factors associated with frequency of recurrence.

	No recurrence (n = 153)[1]	Recurrence (n = 12)	Hazard ratio (95.0% CI)	P value
Past history of IA[2] (%)	24 (15.7)	2 (16.7)	0.914 (0.186–4.478)	0.911
CD4 counts <200[2] (%)	57 (37.3)	3 (25.0)	0.385 (0.101–1.470)	0.162
TPHA test positive[2] (%)	108 (70.6)	10 (83.3)	2.435 (0.501–11.827)	0.270
HBV exposure[2] (%)	92 (60.1)	7 (58.3)	1.248 (0.364–4.277)	0.725
HCV Antibody positive[2] (%)	3 (2.0)	2 (16.7)	7.664 (1.369–42.890)	0.020
Extraluminal disease[2] (%)	66 (43.1)	4 (33.3)	0.559 (0.163–1.921)	0.356
No luminal agent (%)	76 (49.7)	6 (50.0)	1.070 (0.322–3.559)	0.912
Syphilis during follow-up period (%)	33 (21.6)	7 (58.3)	3.332 (0.961–11.547)	0.059

[1]Five patients died within 6 months from disease onset and their data were excluded from analysis.
[2]Status at diagnosis of IA.
doi:10.1371/journal.pntd.0001318.t003

125

that cyst form ameba often disappears spontaneously without any treatment [28,29]. There is controversy about the need for cyst eradication following metronidazole or tinidazole treatment, especially in endemic areas where re-infection is frequent. In this study, recurrence of IA within the first year of metronidazole treatment was noted in only two patients of 82 patients who did not receive luminal therapy. Moreover, long-term follow-up indicated IA recurrence also in those who received luminal agents, and the benefits obtained from luminal treatment seemed to have disappeared. IA recurred more frequently in those with HCV infection, which was recently reported to be transmittable sexually among MSM [30], and in those who acquired new syphilis infection during the follow-up period, suggesting that sexually active MSM tend to experience IA recurrence due to re-acquisition of new *E. histolytica* infection. HBV exposure and positive TPHA at IA diagnosis did not correlate with IA recurrence probably because the high prevalence of these two parameters in this study masked the difference between recurrence and non-recurrence cases. Educational approach for safer sex may be more appropriate rather than luminal treatment to prevent IA recurrence after treatment.

Eleven genetic strains of *E. histolytica* were identified in this study and none of them had been reported so far from geographic areas other than Japan [21,22,31,32], indicating that diverse Japan-specific isolates of *E. histolytica* are already prevalent among MSM in Japan. In fact, the *E. histolytica* seropositivity rate in HIV-1-infected MSM in our clinic was as high as 17.9% in 2009 (unpublished data), which is comparable with the seropositivity rate in Japanese MSM reported more than 20 years ago [5]. Unfortunately, we could not compare the genotypes of *E. histolytica* between the incidences of the primary and recurrent IA within the same individuals due to the lack of appropriate stocked samples, which would have probably demonstrated acquisition of new infection.

Considered together, the results emphasize the difficulty of preventing IA recurrence without educational approach to prevent new amebic infection even after successful IA treatment in the high risk groups such as HIV-1-infected MSM. The spread of *E. histolytica* in MSM of other developed countries beyond Asia should be of great concern.

Supporting Information

Table S1 Patient demographics with and without luminal treatment.
(DOC)

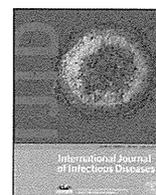**Table S2 Genotyping data of 6 STR loci in 14 clinical samples.**
(DOC)

Author Contributions

Conceived and designed the experiments: HG JT SO. Performed the experiments: KW AEdC TN. Analyzed the data: KW HG. Contributed reagents/materials/analysis tools: KW HG JT SO. Wrote the paper: KW HG.

References

1. Walsh JA (1986) Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality. Rev Infect Dis 8: 228–238.
2. Haque R, Huston CD, Hughes M, Houpt E, Petri WA, Jr. (2003) Amebiasis. N Engl J Med 348: 1565–1573.
3. Takeuchi T, Kobayashi S, Asami K, Yamaguchi N (1987) Correlation of positive syphilis serology with invasive amebiasis in Japan. Am J Trop Med Hyg 36: 321–324.
4. Takeuchi T, Miyahira Y, Kobayashi S, Nozaki T, Motta SR, et al. (1990) High seropositivity for *Entamoeba histolytica* infection in Japanese homosexual men: further evidence for the occurrence of pathogenic strains. Trans R Soc Trop Med Hyg 84: 250–251.
5. Takeuchi T, Okuzawa E, Nozaki T, Kobayashi S, Mizokami M, et al. (1989) High seropositivity of Japanese homosexual men for amebic infection. J Infect Dis 159: 808.
6. Ohnishi K, Kato Y, Imamura A, Fukayama M, Tsunoda T, et al. (2004) Present characteristics of symptomatic *Entamoeba histolytica* infection in the big cities of Japan. Epidemiol Infect 132: 57–60.
7. Ohnishi K, Murata M (1997) Present characteristics of symptomatic amebiasis due to *Entamoeba histolytica* in the east-southeast area of Tokyo. Epidemiol Infect 119: 363–367.
8. Liu CJ, Hung CC, Chen MY, Lai YP, Chen PJ, et al. (2001) Amebic liver abscess and human immunodeficiency virus infection: a report of three cases. J Clin Gastroenterol 33: 64–68.
9. Park WB, Choe PG, Jo JH, Kim SH, Bang JH, et al. (2007) Amebic liver abscess in HIV-infected patients, Republic of Korea. Emerg Infect Dis 13: 516–517.
10. Hung CC, Chen PJ, Hsieh SH, Wong JM, Fang CT, et al. (1999) Invasive amoebiasis: an emerging parasitic disease in patients infected with HIV in an area endemic for amoebic infection. AIDS 13: 2421–2428.
11. Hung CC, Deng HY, Hsiao WH, Hsieh SM, Hsiao CF, et al. (2005) Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. Arch Intern Med 165: 409–415.
12. Hung CC, Ji DD, Sun HY, Lee YT, Hsu SY, et al. (2008) Increased risk for *Entamoeba histolytica* infection and invasive amebiasis in HIV seropositive men who have sex with men in Taiwan. PLoS Negl Trop Dis 2: e175.
13. Chen YM, Kuo SH (2007) HIV-1 in Taiwan. Lancet 369: 623–625.
14. van Griensven F, de Lind van Wijngaarden JW (2010) A review of the epidemiology of HIV infection and prevention responses among MSM in Asia. AIDS 24: S30–40.
15. Lee JH, Kim GJ, Choi BS, Hong KJ, Keo MK, et al. (2010) Increasing late diagnosis in HIV infection in South Korea: 2000-2007. BMC Public Health 10: 411.
16. Annual surveillance report of HIV/AIDS in Japan, 1997 (1999) AIDS Surveillance Committee, Ministory of Health and Welfare, Japan. Working Group of Annual AIDS Surveillance, Ministry of Health and Welfare, Japan. Jpn J Infect Dis 52: 55–87.
17. Tsai JJ, Sun HY, Ke LY, Tsai KS, Chang SY, et al. (2006) Higher seroprevalence of *Entamoeba histolytica* infection is associated with human immunodeficiency virus type 1 infection in Taiwan. Am J Trop Med Hyg 74: 1016–1019.
18. Powell SJ, MacLeod I, Wilmot AJ, Elsdon-Dew R (1966) Metronidazole in amoebic dysentery and amoebic liver abscess. Lancet 2: 1329–1331.
19. Powell SJ, Maddison SE, Elsdon-Dew R (1966) Rapid faecal transmission and invasive amoebiasis in Durban. S Afr Med J 40: 646–649.
20. Hsu MS, Hsieh SM, Chen MY, Hung CC, Chang SC (2008) Association between amebic liver abscess and human immunodeficiency virus infection in Taiwanese subjects. BMC Infect Dis 8: 48.
21. Ali IK, Zaki M, Clark CG (2005) Use of PCR amplification of tRNA gene-linked short tandem repeats for genotyping *Entamoeba histolytica*. J Clin Microbiol 43: 5842–5847.
22. Escueta-de Cadiz A, Kobayashi S, Takeuchi T, Tachibana H, Nozaki T (2010) Identification of an avirulent *Entamoeba histolytica* strain with unique tRNA-linked short tandem repeat markers. Parasitol Int 59: 75–81.
23. Stanley SL, Jr. (2003) Amoebiasis. Lancet 361: 1025–1034.
24. Irusen Em, Jackson TF, Simjee AE (1992) Asymptomatic intestinal colonization by pathogenic *Entamoeba histolytica* in amebic liver abscess: prevalence, response to therapy, and pathogenic potential. Clin Infect Dis 14: 889–893.
25. Blessmann J, Tannich E (2002) Treatment of asymptomatic intestinal *Entamoeba histolytica* infection. N Engl J Med 347: 1384.
26. McAuley JB, Herwaldt BL, Stokes SL, Becher JA, Roberts JM, et al. (1992) Diloxanide furoate for treating asymptomatic *Entamoeba histolytica* cyst passers: 14 years' experience in the United States. Clin Infect Dis 15: 464–468.
27. McAuley JB, Juranek DD (1992) Paromomycin in the treatment of mild-to-moderate intestinal amebiasis. Clin Infect Dis 15: 551–552.
28. Ruiz-Palacios GM, Castanon G, Bojalil R, Tercero E, Rausser S, et al. (1992) Low risk of invasive amebiasis in cyst carriers. A longitudinal molecular seroepidemiological study. Arch Med Res 23: 289–291.
29. Blessmann J, Ali IK, Nu PA, Dinh BT, Viet TQ, et al. (2003) Longitudinal study of intestinal Entamoeba histolytica infections in asymptomatic adult carriers. J Clin Microbiol 41: 4745–4750.
30. van de Laar TJ, Matthews GV, Prins M, Danta M (2010) Acute hepatitis C in HIV-infected men who have sex with men: an emerging sexually transmitted infection. AIDS 24: 1799–1812.
31. Tawari B, Ali IK, Scott C, Quail MA, Berriman M, et al. (2008) Patterns of evolution in the unique tRNA gene arrays of the genus Entamoeba. Mol Biol Evol 25: 187–198.
32. Ali IK, Solaymani-Mohammadi S, Akhter J, Roy S, Gorrini C, et al. (2008) Tissue invasion by Entamoeba histolytica: evidence of genetic selection and/or DNA reorganization events in organ tropism. PLoS Negl Trop Dis 2: e219.

126

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

INTERNATIONAL SOCIETY
FOR INFECTIOUS DISEASES

Trends in early and late diagnosis of HIV-1 infections in Tokyoites from 2002 to 2010

Tsunefusa Hayashida [a,b,c], Hiroyuki Gatanaga [a,c,*], Yukiko Takahashi [a], Fujie Negishi [a], Yoshimi Kikuchi [a], Shinichi Oka [a,c]

[a] AIDS Clinical Center, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan
[b] Japan Foundation for AIDS Prevention, Tokyo, Japan
[c] Center for AIDS Research, Kumamoto University, Kumamoto, Japan

ARTICLE INFO

SUMMARY

Objective: The objective of this study was to delineate the trends in early and late diagnosis of HIV-1 infection in newly diagnosed Tokyoites.
Methods: The BED assay was used to identify cases diagnosed at an early stage of infection. BED-positive non-AIDS cases with a CD4 cell count $\geq 200/\mu l$ were defined as cases with recent infection. The rates of AIDS and recent infection in 809 newly diagnosed Tokyoites during 2002–2010 were analyzed.
Results: The AIDS rate was 22.5%. AIDS patients were older (40.4 years) than non-AIDS patients (35.0 years), and a smaller proportion were men who have sex with men (MSM) in AIDS patients (81.7%) than in non-AIDS patients (89.9%). The AIDS rate was persistently lower ($\leq 14.3\%$) in ≤ 29-year-old than in ≥ 30-year-old MSM. The rate of recent infection was 24.4%. Individuals with recent infection (33.0 years old) were younger than the others (37.2 years). The rate of recent infection was lower ($\leq 18.5\%$) in MSM aged ≥ 40 years than in those aged ≤ 39 years during the study period, except for 2007 and 2008.
Conclusions: Younger MSM Tokyoites appear to be aware of the risk of their sexual behavior, sufficient to take voluntary HIV testing repeatedly, resulting in early diagnosis. Older MSM did not take HIV testing frequently enough and may be a good target for campaigns promoting testing.

1. Introduction

The overall growth of the global AIDS epidemic appears to have stabilized. The annual number of new cases of HIV infection has been in steady decline since the late 1990s.[1] In Japan, however, the annual number of newly diagnosed cases has almost doubled during the most recent decade (791 cases in 2000 and 1544 cases in 2010), although the prevalence of HIV in the adult population remains <0.1%.[2] The distribution of these cases is heavily concentrated in large cities, and approximately 35% of the newly diagnosed cases have been identified in Tokyo.[3]

Early diagnosis of HIV infection is critically important because some AIDS-defining diseases are fatal, even in the era of combination antiretroviral treatment (ART); also the introduction of ART after the development of AIDS is often complicated with immune reconstitution inflammatory syndrome (IRIS).[4,5] In this regard, the introduction of ART at the early stages seems to significantly reduce the sexual transmission of HIV-1.[6,7] Thus, it is important to identify newly infected individuals and provide early ART to reduce the

incidence of AIDS and transmission of HIV. Knowledge about the proportion of patients diagnosed at the early stage of an HIV infection in the newly diagnosed cases is also useful for planning and evaluation of any prevention program and for resource allocation.[8,9] However, it is usually difficult to distinguish recent from long-standing HIV infections except for acute symptomatic infections.[10] Simple prediction of the infection time from CD4 cell counts appears inaccurate because the disease progression rate varies enormously among infected individuals.[11] The BED HIV-1 capture enzyme immunoassay (BED assay) uses the branched peptide to detect HIV-1 IgG antibodies from all subtypes (i.e., HIV-1 B, E, and D gp41 immunodominant sequences are included on a branched peptide used in the assay) and measures levels of anti-HIV-1 IgG relative to total IgG.[12] Since the ratio of anti-HIV-1 IgG to total IgG increases with time shortly after HIV-1 infection, the HIV-1-infected patient is considered to have recently acquired the infection when the normalized optical density (ODn) is less than 0.8 on the BED assay (ODn reaches 0.8 on average 197 days after seroconversion.).[13]

The present study was an attempt to delineate the trends in early diagnosis of HIV-1 infection in Tokyo from 2002 to 2010 by using the BED assay. The aim of this analysis was to enhance our understanding of the status of HIV-1 spread in Tokyo and to help in the design of strategies to control the HIV-1 epidemic in Japan.

* Corresponding author. Tel.: +81 3 3202 7181; fax: +81 3 5273 6483.
  E-mail address: higatana@acc.ncgm.go.jp (H. Gatanaga).

2. Materials and methods

2.1. Newly diagnosed patients

This study included all ART-naïve HIV-1-infected individuals who met the following criteria: (1) those who visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, between 2002 and 2010 within 30 days of their diagnosis with an HIV-1 infection and (2) availability of plasma samples taken at the first visit under signed informed consent for use in viral, immunological, and epidemiological studies. Participant information including CD4 count, HIV-1 load, age at the first visit, gender, nationality, probable HIV-1 transmission route, and history of HIV testing, were collected from the medical records. According to the Japanese law for infection control, physicians are obliged to report newly diagnosed HIV/AIDS cases to the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government). A total of 11 673 HIV/AIDS cases nationally, including 4048 cases diagnosed in Tokyo (Tokyo cases), which were entered into the registry of this committee from 2002 to 2010, were used as the control populations to evaluate the representativeness of the patients enrolled in the present study (AIDS Clinical Center cases).[2,3] Plasma samples obtained from the participants were stored at −80 °C. The viral subtype in each case was determined from the HIV-1 protease–reverse transcriptase sequence (which was analyzed for drug resistance genotyping) by the neighbor-joining method using the Genetic-Win system (Software Development, Tokyo).[14]

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the ethics committee of the National Center for Global Health and Medicine.

2.2. BED assay

The BED HIV-1 capture enzyme immunoassay (BED assay; Calypte Biomedical Corp., Portland, OR, USA) was used to estimate the time of HIV-1 infection.[12] In accordance with the manufacturer's instructions, 5 μl of plasma was diluted with 500 μl of the diluent in the kit, and the proportion of anti-HIV-1-specific IgG to the total IgG in the sample was measured by optical density (OD). The OD values of the test specimens were normalized (ODn) relative to the value of a calibrator (specimen OD/calibrator OD) to minimize inter-run variation. Samples with ODn ≤0.8 were considered to be from individuals who had seroconverted within 197 days and were defined as BED-positive.[13] BED-positive non-AIDS cases with CD4 cell counts ≥200/μl were defined as individuals with recent infection. The others were defined as chronic infection.

2.3. Statistical analysis

Differences in demographic data including age, gender, risk behavior, nationality, and AIDS development among the AIDS Clinical Center cases, national cases, and Tokyo cases, were examined for significance using one-way analysis of variance (ANOVA) and the Tukey test, or Pearson's Chi-square test. Differences in demographic data including age, CD4 count, logarithmic HIV-1 viral load, nationality, transmission category, HIV-1 subtype, cue for HIV diagnosis, and history of HIV testing, between AIDS and non-AIDS patients and between recent and chronic infection, were examined for significance using the t-test or Pearson's Chi-square test. To estimate the correlation with the development of AIDS, binominal logistic regression analysis including age, nationality (Japanese or not), and transmission category (men having sex with men (MSM) or not) was performed. A p-value of less than 5% denoted statistical significance. Statistical

analyses were performed with SPSS Statistics 17.0 (IBM Japan Inc., Tokyo, Japan) and Stat Mate II (NANKODO, Tokyo).

3. Results

3.1. Newly diagnosed cases of HIV-1 infection

The study subjects were 809 ART-naïve HIV-1-infected patients. All of them had visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, within 30 days of the diagnosis of HIV-1 infection (median 8 days) between 2002 and 2010. They included 741 Japanese, 35 Asians other than Japanese, and 33 from other countries. They represented 20.0% of the total number of newly diagnosed Tokyoite cases during the same period (Table 1). There were no significant differences in the proportion of AIDS (22.5% vs. 21.9%), percentage of males (96.2% vs. 94.3%), or proportion of Japanese (91.6% vs. 90.7%) between our study patients and those of the Tokyo registry, although our patients included a significantly smaller proportion of AIDS cases (22.5% vs. 30.4%) and significantly larger population of male patients (96.2% vs. 91.8%) and Japanese patients (91.6% vs. 88.5%) compared with the patients of the national registry. Furthermore, our patients were significantly younger than the patients of the Tokyo and national registries (36.2 vs. 37.7 and 38.0 years), and the proportion of MSM among male patients was significantly higher than in the Tokyo and national registries (88.0% vs. 72.8% and 59.8%).

Subtype analysis successfully determined the HIV-1 subtype in 807 patients (99.8%); the majority were infected with HIV-1 subtype B (742 patients, 91.9%), while 5.7% were infected with HIV-1 subtype AE, which is comparable to previously published subtype data in Japan.[14] The HIV-1 subtype could not be determined in two patients because the viral load was below the detection limit (<40 copies/ml), although they were not being treated with anti-HIV drugs.

3.2. Features of AIDS patients

Among the 809 cases, 182 (22.5%, 95% confidence interval (95% CI) 19.6–25.4) had already developed AIDS at the first visit, while the other 627 were non-AIDS cases (Table 2). AIDS cases were significantly older (40.4 years, 95% CI 38.8–41.9 vs. 35.0 years, 95% CI 34.2–35.9), and as expected, had lower CD4 counts (61.7/μl, 95% CI 50.6–72.8 vs. 318.0/μl, 95% CI 303.0–333.0) and higher viral loads (5.22 log VL/ml, 95% CI 5.13–5.31 vs. 4.63 log VL/ml, 95% CI 4.56–4.70) than non-AIDS patients. There were no significant differences in nationality (Japanese 91.8%, 95% CI 87.8–95.8 vs. 91.5%, 95% CI 89.4–93.7) or HIV-1 subtype (subtype B 89.0%, 95% CI 84.5–93.6 vs. 92.5%, 95% CI 90.4–94.6) between AIDS and non-AIDS

Table 1
New cases of HIV-1-infected patients diagnosed between 2002 and 2010

	Japan[a]	Tokyo[b]	This study
Number of cases	11 673	4048	809
Age, years (mean ± SD)	38.0 ± 11.8[c]	37.7 ± 11.9[d]	36.2 ± 11.0
Males	10 721 (91.8%)[c]	3819 (94.3%)	778 (96.2%)
Men having sex with men	6408 (59.8%)[c]	2780 (72.8%)[c]	685 (88.0%)
Japanese	10 335 (88.5%)[d]	3673 (90.7%)	741 (91.6%)
AIDS cases	3551 (30.4%)[c]	885 (21.9%)	182 (22.5%)

Statistical analyses were performed by one-way ANOVA and Tukey test, or Chi-square test.
[a] Provided by the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government).
[b] Provided by the Bureau of Social Welfare and Public Health, Tokyo.
[c] p < 0.001, compared with the study participants.
[d] p < 0.01 compared with the study participants.

Table 2
Demographics of participants with and without AIDS

	AIDS (n = 182)		Non-AIDS (n = 627)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	40.4	(38.8–41.9)	35.0	(34.2–35.9)	<0.001
CD4 count /μl	61.7	(50.6–72.8)	318.0	(303.0–333.0)	<0.001
Log viral load/ml	5.22	(5.13–5.31)	4.63	(4.56–4.70)	<0.001
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.424
Japan	167	91.8 (87.8–95.8)	574	91.5 (89.4–93.7)	
Asia other than Japan	11	6.0 (3.3–10.8)	24	3.8 (2.6–5.7)	
North and South America	2	1.1 (0.2–4.0)	17	2.7 (1.7–4.3)	
Africa	2	1.1 (0.2–4.0)	6	1.0 (0.4–2.1)	
East and West Europe	0	0 (0–2.0)	4	0.6 (0.2–1.6)	
Oceania	0	0 (0–2.0)	2	0.3 (0–1.1)	
Transmission category					
Male	175	96.2 (93.4–98.9)	603	96.2 (94.7–97.7)	0.024
MSM	143	81.7 (76.0–87.4)	542	89.9 (87.5–92.3)	
Heterosexual	21	12.0 (7.2–16.8)	43	7.1 (5.4–9.6)	
IDU	1	0.6 (0–3.2)	2	0.3 (0.1–1.2)	
Unknown	10	5.7 (3.0–10.5)	16	2.7 (1.6–4.3)	
Female	7	3.8 (1.7–7.9)	24	3.8 (2.6–5.7)	–
Heterosexual	7	100 (46.8–100)	24	100 (100–100)	
Subtype					0.351
B	162	89.0 (84.5–93.6)	580	92.5 (90.4–94.6)	
AE	16	8.8 (5.4–14.3)	30	4.8 (3.4–6.8)	
C	1	0.5 (0–3.0)	7	1.1 (0.5–2.3)	
G	2	1.1 (0.2–4.0)	3	0.5 (0.1–1.4)	
AG	1	0.5 (0–3.0)	3	0.5 (0.1–1.4)	
A	0	0 (0–2.0)	2	0.3 (0–1.1)	
Unknown	0	0 (0–2.0)	2	0.3 (0–1.1)	
Cue for HIV diagnosis					<0.001
Voluntary testing	12	6.6 (3.7–11.5)	283	45.1 (41.2–49.0)	
Provider-initiated testing	167	91.8 (87.8–95.8)	338	53.9 (50.0–57.8)	
Unknown	3	1.6 (0.4–4.8)	6	1.0 (0.4–2.1)	
Previous testing					<0.001
Yes	29	15.9 (10.6–21.3)	282	45.0 (41.1–48.9)	
No	65	35.7 (28.8–42.7)	254	40.5 (36.7–44.4)	
Unknown	88	48.4 (41.1–55.6)	91	14.5 (11.8–17.3)	
BED assay					<0.001
Recent (ODn ≤0.8)	47	25.8 (19.5–32.2)	255	40.7 (36.8–44.5)	
Chronic (ODn >0.8)	135	74.2 (67.8–80.5)	372	59.3 (55.5–63.2)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user; ODn, normalized optical density.
[a] By t-test or Pearson's Chi-square test.

cases (Pearson's Chi-square test). MSM activity was the most frequent transmission route in both groups, and still more frequent in non-AIDS cases (89.9%, 95% CI 87.5–92.3) than in AIDS cases (81.7%, 95% CI 76.0–87.4). A larger proportion of patients in the non-AIDS group than in the AIDS group had undertaken previous HIV testing (45.0%, 95% CI 41.1–48.9 vs. 15.9%, 95% CI 10.6–21.3) and had been diagnosed with HIV-1 infection by voluntary testing (45.1%, 95% CI 41.2–49.0 vs. 6.6%, 95% CI 3.7–11.5), suggesting that repeated voluntary testing may prevent disease progression to AIDS in the high-risk groups.

Binominal logistic regression analysis of age, nationality (Japanese or not), and transmission category (MSM or not) identified age as the most significant factor associated with the development of AIDS (per 1-year increment, (hazard ratio) HR 1.041, 95% CI 1.026–1.057; $p < 0.001$).

To delineate the trends in late diagnosis of HIV-1 infection, the annual rates of AIDS cases in newly-diagnosed HIV-1-infected patients were plotted through the study period. The rate of AIDS cases remained around 30% between 2002 and 2004. It decreased to 15.0% in 2005, but then showed a gradual increase annually, reaching 24.8% in 2010 (Figure 1). To identify the population that influenced the increase in the rate of AIDS cases in the most recent years, we selected and categorized the study participants based on their features. Specifically, we focused on MSM patients, because 85% of our patients were MSM. Based on the above results of the

significance of age in the binominal logistic regression analysis in the development of AIDS, we examined the effect of age in more detail by dividing the MSM patients into three age groups: those aged ≤29 years (217 patients, 31.7%), 30–39 years (273 patients, 39.9%), and ≥40 years (195 patients, 28.5%). In the ≥40 years MSM group, the rate was higher than 50% between 2002 and 2004, but decreased to 21.4% in 2005 and further decreased to 14.3% in 2006, but gradually increased and reached ~30% in 2009 and 2010 (Figure 1). On the other hand, in the ≤29 years MSM group, the AIDS rate was steadily lower than 20%, indicating that most young HIV-1-infected MSM were diagnosed before the development of AIDS throughout the study period. The AIDS rate in the 30–39 years MSM group was between those of the other two groups during most of the study period. A significantly larger proportion of patients in the ≤29 years MSM group had undergone voluntary HIV testing (43.8%, $p = 0.002$, Pearson's Chi-square test) and diagnosis with HIV (48.8%, $p < 0.001$, Pearson's Chi-square test), compared with the 30–39 years MSM group (43.6% and 36.6%, respectively) and the ≥40 years MSM group (34.9% and 32.3%, respectively). These results suggest that repeated voluntary testing may have prevented disease progression to AIDS in the younger MSM groups. The high rate of AIDS in all the study participants observed in 2002–2004 seemed mainly due to the ≥40-year-old MSM. Furthermore, the gradual increase in the AIDS rate in the ≥40-year-old MSM since 2006 also seemed to have contributed to
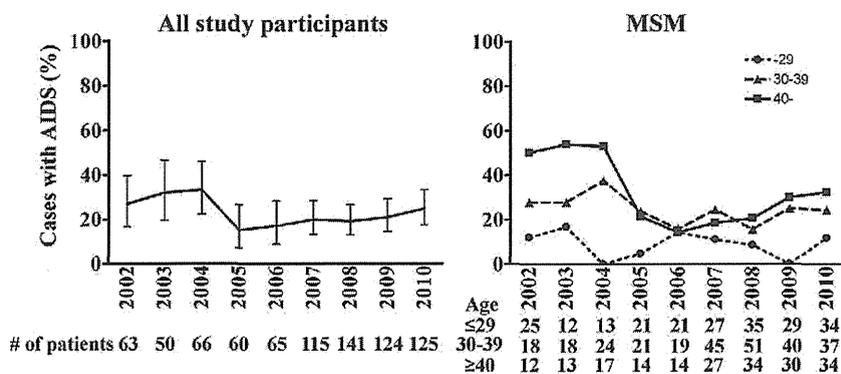
Figure 1. Annual rate of AIDS in newly diagnosed HIV-1-infected individuals. The annual AIDS rate for all study participants (809 patients; left panel), and men who have sex with men (MSM) categorized by age: ≤29 years (n = 217), 30–39 years (n = 273), and ≥40 years (n = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S1).

the rising AIDS rate in all, suggesting that older MSM should be the main target for interventions aimed at promoting HIV testing for early diagnosis and prevention of the development of AIDS.

3.3. Trends in early HIV diagnosis

To identify individuals with recent HIV-1 infection, we performed a BED assay for the 809 study participants. Before analysis of the results, we dealt with the problem of potential

misclassification. Previous studies reported small levels of anti-HIV-1-specific IgG relative to the total IgG in cases with both recent HIV-1 infection and long-standing chronic cases with severe immunodeficiency, which could result in false classification of chronic cases as recent infection.[12,15,16] To tackle this problem, previous studies classified AIDS cases and cases with CD4 cell counts <200/μl as chronic infection cases, in accordance with the Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) guidelines.[17–21] We applied

Table 3
Demographics of participants with recent and chronic infection

	Recent (n = 197)		Chronic (n = 612)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	33.0	(31.7–34.3)	37.2	(36.3–38.1)	<0.001
CD4 count /μl	423.2	(399.2–447.3)	207.9	(193.3–222.4)	<0.001
Log viral load/ml	4.61	(4.46–4.76)	4.81	(4.74–4.87)	0.005
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.101
Japan	189	95.9 (93.2–98.7)	552	90.2 (87.8–92.6)	
Asia other than Japan	2	1.0 (0.2–3.7)	33	5.4 (3.9–7.6)	
North and South America	3	1.5 (0.4–4.4)	16	2.6 (1.6–4.2)	
Africa	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
East and West Europe	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
Oceania	1	0.5 (0–2.8)	1	0.2 (0–0.9)	
Transmission category					
Male	192	97.5 (95.3–99.7)	586	95.8 (94.2–97.3)	0.314
MSM	177	92.2 (88.4–96.0)	508	86.7 (83.9–89.4)	
Heterosexual	11	5.7 (3.1–10.2)	53	9.0 (7.0–11.8)	
IDU	0	0 (0–1.9)	3	0.5 (0.1–1.5)	
Unknown	4	2.1 (0.7–5.3)	22	3.8 (2.5–5.7)	
Female	5	2.5 (1.0–5.9)	26	4.2 (2.9–6.2)	–
Heterosexual	5	100 (34.4–100)	26	100 (81.5–100)	
Subtype					0.029
B	188	95.4 (92.5–98.3)	554	90.5 (88.2–92.8)	
AE	4	2.0 (0.7–5.2)	42	6.9 (5.2–9.3)	
C	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
G	1	0.5 (0–2.8)	4	0.7 (0.2–1.7)	
AG	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
A	0	0 (0–1.9)	2	0.3 (0–1.2)	
Unknown	2	1.0 (0.2–3.7)	0	0 (0–0.6)	
Cue for HIV diagnosis					<0.001
Voluntary testing	102	51.8 (44.8–58.8)	193	31.5 (27.9–35.2)	
Provider-initiated testing	94	47.7 (40.7–54.7)	411	67.2 (63.4–70.9)	
Unknown	1	0.5 (0–2.8)	8	1.3 (0.6–2.6)	
Previous testing					<0.001
Yes	116	58.9 (52.0–65.8)	195	31.9 (28.2–35.6)	
No	57	28.9 (22.6–35.3)	262	42.8 (38.9–46.7)	
Unknown	24	12.2 (7.6–16.8)	155	25.3 (21.9–28.8)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user.
[a] By t-test or Pearson's Chi-square test.

| # of patients | 63 | 50 | 66 | 60 | 65 | 115 | 141 | 124 | 125 |

Age	2002	2003	2004	2005	2006	2007	2008	2009	2010
≤29	25	12	13	21	21	27	35	29	34
30-39	18	18	24	21	19	45	51	40	37
≥40	12	13	17	14	14	27	34	30	34
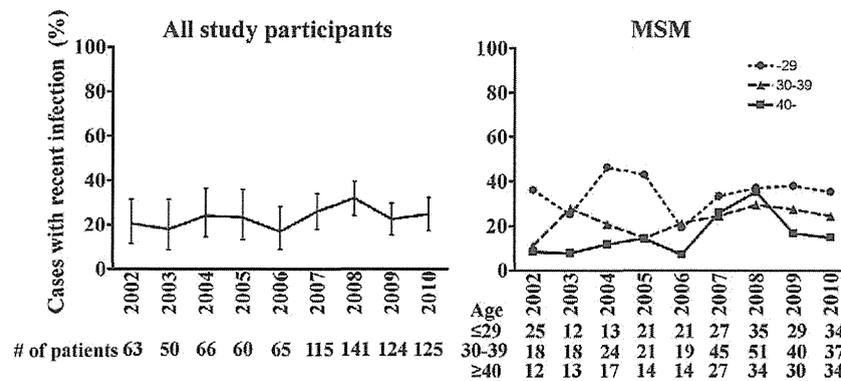
Figure 2. Annual rate of recent infection in newly diagnosed HIV-1-infected cases. The annual rate of recent infection in all study participants (809 patients; left panel), and in men who have sex with men (MSM) categorized by age: ≤29 years (*n* = 217), 30–39 years (*n* = 273), and ≥40 years (*n* = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S2).

the same strategy in this study and thus defined only BED-positive non-AIDS cases with CD4 cell counts ≥200/μl as recent infection.

In the 456 non-AIDS cases with CD4 cell counts ≥200/μl, 197 cases were BED-positive and classified as recent infection (43.2%; 24.4% of the total cases) (Table 3). BED-negative cases, AIDS cases, and cases with CD4 cell counts <200/μl were classified as chronic infection. Patients with recent infection were younger (33.0 years, 95% CI 31.7–34.3 vs. 37.2 years, 95% CI 36.3–38.1) and had higher CD4 counts (423.2/μl, 95% CI 399.2–447.3 vs. 207.9/μl, 95% CI 193.3–222.4), as expected, and lower viral load (4.61 log VL/ml, 95% CI 4.46–4.76 vs. 4.81 log VL/ml, 95% CI 4.74–4.87), compared to patients with chronic infection. A larger proportion of recent infection (95.4%, 95% CI 92.5–98.3) was caused by HIV-1 subtype B than in those with chronic infection (90.5%, 95% CI 88.2–92.8). There were no significant differences in the nationality and transmission category between recent and chronic infection cases (Pearson's Chi-square test), although the proportion of Japanese patients was higher in recent infection (95.9%, 95% CI 93.2–98.7) than in chronic infection (90.2%, 95% CI 87.8–92.6) (*p* = 0.012, Chi-square test). A significantly larger proportion of patients underwent previous HIV testing (58.9%, 95% CI 52.0–65.8 vs. 31.9%, 95% CI 28.2–35.6) and were diagnosed with HIV-1 infection by voluntary testing (51.8%, 95% CI 44.8–58.8 vs. 31.5%, 95% CI 27.9–35.2) among recent infection cases than chronic infection cases (*p* < 0.001 in both, Pearson's Chi-square test).

To delineate the trends in early diagnosis of HIV-1 infection, the annual rate of recent infection in all 809 study participants was plotted over the study period (Figure 2). The rate was stable at ~20% between 2002 and 2010, except for 2007 (26.1%) and 2008 (31.9%), when a slight increase was evident. In order to identify the population that influenced the annual trends of early diagnosis, we focused on MSM patients and again divided them into three age groups: ≤29 years, 30–39 years, and ≥40 years. The rates of recent infection in the ≤29 and ≥40 years MSM groups were the highest and the lowest, respectively, in most years of the study period. The rate in the ≤29 years MSM group was high, ranging from 25.0% to 46.2% between 2002 and 2005, but it decreased to 19.0% in 2006, and increased again in 2007 and remained around 35% between 2007 and 2010. The rate of recent infection in the ≥40-year-old MSM group was steadily low at ~10% between 2002 and 2006, but increased in 2007 to 25.9% and 2008 to 35.3%, then decreased to around 15% in 2009 and 2010. The rate in the 30–39-year-old MSM ranged between those of the other two groups during most part of the study period. These results suggest that younger MSM tend to be diagnosed persistently earlier, whereas older MSM are usually diagnosed at a later stage of the HIV disease.

4. Discussion

The present study analyzed the trends in the proportion of AIDS patients and patients with recent infection among 809 new cases of HIV-1-infection diagnosed between 2002 and 2010. This group recruited from our AIDS Clinical Center represents 20.0% of the total number of newly diagnosed Tokyoites during the same period. We found that MSM, especially younger MSM, tend to be diagnosed at an earlier stage before the development of AIDS, probably because of frequent voluntary HIV testing. The proportion of AIDS cases remained at a steady low level and the rate of recent infection remained at a high level in younger MSM patients, indicating that younger MSM are aware of the risk of their sexual behavior sufficient to take HIV testing repeatedly. On the other hand, in the older MSM, the rate of AIDS was relatively high and the rate of recent infection comparatively low, but transiently increased in 2007 and 2008, suggesting that older MSM with a high-risk of HIV infection usually do not take HIV testing frequently and may respond to campaigns that promote such tests. Interestingly, the Japan Foundation for AIDS Prevention conducted several campaigns to promote voluntary HIV-1 testing in 2007. A popular male Japanese singer took part in one such campaign in July 2007, which was a great surprise among the Japanese in general, and this was followed by an increase in the number of voluntary HIV tests performed in 2007 and 2008.[2] The event may have prompted older MSM at high risk to take voluntary HIV testing, resulting in the transient increase in the rate of early diagnosis for 2007 and 2008. The sharp decline in the rate of early diagnosis observed in 2009 and 2010 in the older MSM group coincided with reductions in the number of voluntary tests,[2] and could be an omen of future increases in the number of AIDS patients in this population. Early diagnosis followed by early introduction of ART may reduce the spread of HIV-1 among MSM, which could help to prevent an HIV epidemic in this population.[6,7,22] A strategy based on the promotion of voluntary testing needs to be formulated, similar to the 2007 campaigns that resulted in significant increases in the rate of early diagnosis in older MSM.

Discordant shifts were observed between the rates of AIDS and recent infection. The reasons may be that AIDS usually develops several years after HIV infection and that disease progression varies enormously among infected individuals. Therefore, the variable length of time during which HIV infection was ignored resulted in the development of AIDS, the proportion of which does not always correlate with the rate of recent infection in the same year.[11] Furthermore, disease progression has been suggested to have become faster in a significant portion of Japanese patients, probably because the prevailing HIV-1 strains in Japan have

adapted to the Japanese population by acquiring escape mutations from immune pressure restricted by human leukocyte antigens (HLAs) popular among the Japanese.[23,24] Based on this point of view, early diagnosis is even more important due to the shorter asymptomatic period before the development of AIDS.

The majority of our study participants were infected with HIV-1 subtype B, and HIV-1 subtype B infection correlated significantly with MSM (crude odds ratio 37.9, $p < 0.001$; Chi-square test). The non-AIDS patients were more likely to be infected with subtype B than AIDS patients (crude odds ratio 1.59, $p = 0.098$). The same was true for recent infection than chronic infection (crude odds ratio 2.81, $p = 0.009$). A previous Japan-wide survey also showed a close relationship between subtype B and MSM in Japan; all cases diagnosed with primary HIV-1 infection ($n = 45$) were caused by subtype B, and such primary infections were significantly frequent among MSM.[14] Considered together, the results indicate that subtype B is the major currently prevalent strain in Japan, especially among MSM, and such strains are probably adapting to the Japanese population by repeated exposure to immune pressure of the Japanese.

This study used case reporting-based surveillance to estimate the number of new HIV-1 infections in Tokyoites between 2002 and 2010. The data were collected at a single center and thus may have included some institutional bias. The study participants were statistically younger and were more likely to be MSM than those of the Tokyo registry. The BED assay was used in this study to determine the rate of recent infection in the selection study group and not to determine the national incidence rate. However, the data from this study suggest the following target-specific differential strategies for controlling the HIV epidemic and for AIDS prevention in Tokyo: campaigns aimed at promoting testing should be directed at older MSM for early diagnosis to prevent/halt the progression of AIDS; commencement of ART for HIV-infected younger MSM at early stages of the disease may effectively reduce the number of new cases based on the control of current hot-spots of HIV transmission among this group.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2011.11.003.

References

1. Global report: UNAIDS report on the global AIDS epidemic 2010. UNAIDS; 2010. Available at: http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf (accessed on 2 November 2011).
2. AIDS Prevention Information Network (API-Net). Comment of National AIDS Surveillance Committee, Japan, 2011. API-Net. Available at: http://api-net.jfap.or.jp/status/index.html (accessed on 2 November 2011).
3. Bureau of Social Welfare and Public Health, Tokyo. AIDS Newsletter No. 135, 2011. Bureau of Social Welfare and Public Health. Available at: http://www.fukushihoken.metro.tokyo.jp/iryo/kansen/aids/newsletter/files/AIDS_News_Letter_No.135-1.pdf (accessed on 2 November 2011).
4. Müller M, Wandel S, Colebunders R, Attia S, Furrer H, Egger M, et al. Immune reconstitution inflammatory syndrome in patients starting antiretroviral therapy for HIV infection: a systemic review and meta-analysis. *Lancet Infect Dis* 2010;**10**:251–61.
5. Lipman M, Breen R. Immune reconstitution inflammatory syndrome in HIV. *Curr Opin Infect Dis* 2006;**19**:20–5.
6. Donnell D, Baeten JM, Kiarie J, Thomas KK, Stevens W, Cohen CR, et al. Heterosexual HIV-1 transmission after initiation of antiretroviral therapy: a prospective cohort analysis. *Lancet* 2010;**375**:2092–8.
7. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011;**365**:493–505.
8. Murillo W, Paz-Bailey G, Morales S, Monterroso E, Paredes M, Dobbs T, et al. Transmitted drug resistance and type of infection in newly diagnosed HIV-1 individuals in Honduras. *J Clin Virol* 2010;**49**:239–44.
9. Kao CF, Chang SY, Hsia KT, Chang FY, Yang CH, Liu HR, et al. Surveillance of HIV type 1 recent infection and molecular epidemiology among different risk behaviors between 2007 and 2009 after the HIV type 1 CRF07_BC outbreak in Taiwan. *AIDS Res Hum Retroviruses* 2011;**27**:745–9.
10. Cohen MS, Gay CL, Busch MP, Hecht FM. The detection of acute HIV infection. *J Infect Dis* 2010;**202**:S270–7.
11. Casado C, Colombo S, Rauch A, Martinez R, Gunthard HF, Garcia S, et al. Host and viral genetic correlates of clinical definitions of HIV-1 disease progression. *PLoS One* 2010;**5**:11079.
12. Parekh BS, Kennedy MS, Dobbs T, Pau CP, Byers R, Green T, et al. Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. *AIDS Res Hum Retroviruses* 2002;**18**:295–307.
13. Parekh BS, Hanson DL, Hargrove J, Branson B, Green T, Dobbs T, et al. Determination of mean recency period for estimation of HIV type 1 incidence with the BED-capture EIA in persons infected with diverse subtypes. *AIDS Res Hum Retroviruses* 2011;**27**:265–73.
14. Gatanaga H, Ibe S, Matsuda M, Yoshida S, Asagi T, Kondo M, et al. Drug-resistant HIV-1 prevalence in patients newly diagnosed with HIV/AIDS in Japan. *Antiviral Res* 2007;**75**:75–82.
15. Le Vu S, Pillonel J, Semaille C, Bernillon P, Le Strat Y, Meyer L, et al. Principles and uses of HIV incidence estimation from recent infection testing—a review. *Euro Surveill* 2008;**13**:11–6.
16. Marinda ET, Hargrove J, Preiser W, Slabbert H, van Zyl G, Levin J, et al. Significantly diminished long-term specificity of the BED capture enzyme immunoassay among patients with HIV-1 with very low CD4 counts and those on antiretroviral therapy. *J Acquir Immune Defic Syndr* 2010;**53**:496–9.
17. Jiang Y, Wang M, Ni M, Duan S, Wang Y, Feng J, et al. HIV-1 incidence estimates using IgG-capture BED-enzyme immunoassay from surveillance sites of injection drug users in three cities of China. *AIDS* 2007;**21**:S47–51.
18. Hall HI, Song R, Rhodes P, Prejean J, An Q, Lee LM, et al. Estimation of HIV incidence in the United States. *JAMA* 2008;**300**:520–9.
19. Scheer S, Chin CS, Buckman A, McFarland W. Estimation of HIV incidence in San Francisco. *AIDS* 2009;**23**:533–4.
20. Duan S, Shen S, Bulterys M, Jia Y, Yang Y, Xiang L, et al. Estimation of HIV-1 incidence among five focal populations in Dehong, Yunnan: a hard hit area along a major drug trafficking route. *BMC Public Health* 2010;**10**:180.
21. UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance. When and how to use assays for recent infection to estimate HIV incidence at a population level. Geneva: WHO; 2011. Available at: http://www.who.int/hiv/pub/surveillance/sti_surveillance/en/index.html (accessed on 2 November 2011).
22. Fisher M, Pao D, Brown AE, Sudarshi D, Gill ON, Cane P, et al. Determinants of HIV-1 transmission in men who have sex with men: a combined clinical, epidemiological and phylogenetic approach. *AIDS* 2010;**24**:1739–47.
23. Nakamura H, Teruya K, Takano M, Tsukada K, Tanuma J, Yazaki H, et al. Clinical symptoms and courses of primary HIV-1 infection in recent years in Japan. *Intern Med* 2011;**50**:95–101.
24. Kawashima Y, Pfafferott K, Frater J, Matthews P, Payne R, Addo M, et al. Adaptation of HIV-1 to human leukocyte antigen class I. *Nature* 2009;**458**:641–5.

Correspondence

Reply to 'pharmacokinetic concerns related to darunavir/ritonavir plus raltegravir combination therapy trial'

Gervasoni and Cattaneo [1] point out that a potential pharmacokinetic drug–drug interaction between raltegravir and darunavir ultimately affecting the results of the AIDS Clinical Trials Group (ACTG) A5262 trial cannot be ruled out. We agree with this statement, as A5262 was never designed to be a drug–drug interaction study. Indeed, we refrained from categorically rejecting the possibility of significant interactions and simply presented our finding that trough concentrations observed in A5262 were within the range reported in an intensive pharmacokinetic study of darunavir 800/100 mg daily [2].

In A5262, the darunavir troughs were different in the nonvirologic failure (1649 ng/ml) and virologic failure groups (1042 ng/ml), and undetectable raltegravir trough concentrations were associated with increased virologic failure. Importantly, A5262 was not designed to elicit optimal drug exposure variables, nor was it our intention to use the results as a basis for therapeutic drug monitoring (TDM). In applying TDM, the therapeutic range of the drug must first be defined, and this has not been adequately done for raltegravir or darunavir. Along this line, the suggestion by Gervasoni and Cattaneo [1] that darunavir area under the curve (24 h) is the relevant pharmacokinetic parameter raises important questions. What is the optimum exposure of darunavir in HIV-infected patients? Are troughs more important or is the area under the curve? Is there consensus on this exposure variable, and were those data generated from well designed dose-ranging studies or retrospective pharmacokinetic cohort analyses? Clearly, these issues are yet to be resolved in the literature. Without knowing what the ideal darunavir exposure target is, neither our team nor Gervasoni and Cattaneo [1] can state whether virologic failure occurred due to low darunavir troughs, regardless of whether raltegravir caused low darunavir exposure or not. There are in-vitro data to suggest the darunavir protein-binding-corrected 95% inhibitory concentration might be as low as 25 ng/ml [3], which is well below the darunavir trough concentrations in both virologic failure and nonvirologic failure participants in A5262. Previous pharmacokinetic studies of once-daily darunavir found no relevant relationships between darunavir pharmacokinetics and virologic efficacy or safety [4,5].

Gervasoni and Cattaneo [1] also state that no association between raltegravir pharmacokinetics and clinical outcome can be reasonably expected. They apparently based this assertion on raltegravir's pharmacokinetic variability and long residence time on the pre-integration complex, which exceeds the half-life of the complex itself. This presumably makes raltegravir inhibition of the enzyme complex irreversible, which was extrapolated to explain why raltegravir troughs are not related to response. According to Gervasoni and Cattaneo [1], it is unlikely that raltegravir trough concentrations can *per se* directly affect response to therapy of patients enrolled in the ACTG trial. Therefore, they posited that other ways in which raltegravir could indirectly impact on patient outcome, such as an interaction with darunavir, should be advocated. Although there may be a drug–drug interaction, it should be emphatically reiterated that A5262 was not designed to assess this. More importantly, the phase III study of the safety and efficacy of once daily versus twice daily raltegravir in combination therapy for treatment-naïve HIV-infected patients (QDMRK) clearly showed that raltegravir 800 mg once daily was inferior to 400 mg twice daily at 48 weeks [6]; the geometric mean trough concentration$_h$ was 83 nmol/l for once-daily vs. 380 nmol/l for twice-daily dosing. The QDMRK study was a large dose fractionation study, and as such we now know that the raltegravir trough concentrations are indeed an important determinant of therapeutic response. The half-life of drug binding to the integration complex may be an important variable for certain dosing intervals but, if the dosing interval exceeds some threshold, then the integration complex can form and integrate during the period when raltegravir concentrations are low or absent. This is further demonstrated by data with dolutegravir (same mechanism of action as raltegravir), which exhibited a concentration–response relationship when a wide range of doses (25-fold) was used in early development [7]. A recent article demonstrates the dissociative half-life of dolutegravir is 71 h, considerably longer than the 8.8 h for raltegravir [8]. Thus, a concentration–response relationship was still determined for dolutegravir, even though the enzyme complex off-rate was eight times slower. Gervasoni and Cattaneo [1] are incorrect in assuming that the raltegravir trough concentrations do not affect response to therapy in A5262, and we cannot confirm nor rule out a drug–drug interaction between darunavir and raltegravir in this particular study.

Acknowledgements

Edward P. Acosta[a], Babafemi Taiwo[b] and Joseph J. Eron[c], [a]Division of Clinical Pharmacology, Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, Alabama, [b]Division of Infectious Diseases, Northwestern University, Chicago, Illinois, and [c]Division of Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.

Correspondence to Babafemi Taiwo, MBBS, Division of Infectious Diseases, Northwestern University, Chicago, IL 60611, USA.
Tel: +1 312 695 5085; fax: +1 312 695 5088;
e-mail: b-taiwo@northwestern.edu

References

1. Gervasoni C, Cattaneo D. **Pharmacokinetic concerns related to the ACTG A5262 trial.** AIDS 2012; **26:**398–400.
2. Boffito M, Moyle M, Hill A, Sekar V, Lefebvre E, De Pauw M, et al. **The pharmacokinetic profile of darunavir with low-dose ritonavir (DRV/r) in various multiple-dose regimens over 120 h** [abstract P31]. In: 9th International Workshop on Clinical Pharmacology of HIV Therapy; 7–9 April 2008; New Orleans, Louisiana, USA.
3. Acosta EP, Limoli KL, Trinh L, Parkin NT, King JR, Weidler J, et al. **Novel method to assess antiretroviral target trough concentrations using in vitro susceptibility data (N-145).** In: 18th Conference on Retroviruses and Opportunistic Infections; 27 February to 2 March 2011; Boston, Massachusetts, USA.
4. Sekar V, Abeele VC, Van Baelen B, Vis P, Lavreys L, De Pauw M, et al. **Pharmacokinetic-pharmacodynamic (PK/PD) analyses of once-daily darunavir in the ARTEMIS study** [poster 42]. In: 9th International Workshop on Clinical Pharmacology of HIV Therapy; 7–9 April 2008; New Orleans, Louisiana, USA.
5. Sekar V, De La Rosa G, Van de Casteele T, Spinosa-Guzman S, Vis P, Hoetelmans RMW. **Pharmacokinetic (PK) and pharmacodynamic analyses of once- and twice-daily darunavir/ritonavir (DRV/r) in the ODIN trial. Poster 185.** In: 10th International Conference on Drug Therapy in HIV Infection; 7–11 November 2010; Glasgow, UK.
6. Wenning L, Rizk M, Luo W, Hang Y, Su J, Campbell H, et al. **PK/PD analyses for QDMRK: a phase 3 study of the safety and efficacy of once versus twice daily raltegravir in treatment-naïve HIV-infected patients** [oral abstract O_09]. In: 12th International Workshop on Clinical Pharmacology of HIV Therapy; 13–15 April 2011; Miami, Florida, USA.
7. Min S, Song I, Borland J, Chen S, Lou Y, Fujiwara T, et al. **Pharmacokinetics and safety of S/GSK1349572, a next-generation HIV integrase inhibitor, in healthy volunteers.** Antimicrob Agents Chemother 2010; **54:**254–258.
8. Hightower KE, Wang R, Deanda F, Johns BA, Weaver K, Shen Y, et al. **Dolutegravir (S/GSK 1349572) exhibits significantly slower dissociation than raltegravir and elvitegravir from wild-type and integrase inhibitor-resistant HIV-1 integrase.** Antimicrob Agents Chemother 2011; **55:**4552–4559.

Pharmacokinetic concerns related to the AIDS Clinical Trial Group (ACTG) A5262 trial

Taiwo et al. [1] have recently reported results of the AIDS Clinical Trial Group (ACTG) A5262 trial, specifically designed to investigate a two-drug, reverse transcriptase inhibitor-sparing regimen of darunavir/ritonavir (DRV/r) with raltegravir (RAL) for initial antiretroviral therapy. The proposed regimen met the protocol definition of 'acceptable virologic efficacy', but only 60% of participants reached viral load less than 50 copies/ml at week 48 in spite of an unanticipated high incidence of virologic failure and integrase resistance especially in patients with baseline viral load more than 100 000 copies/ml.

Taiwo et al. [1] have attempted to find out potential explanations for these unexpected results. Particularly, they have explored the potential contribution of DRV and RAL pharmacokinetics on the study findings. Average DRV and RAL trough concentrations were not significantly different for patients with and without virologic failure. However, sensitivity analyses evidenced a significant role of DRV levels, which were significantly lower in patients with virologic failure compared with those without virologic failure (1042 vs. 1649 ng/ml, $P = 0.017$). This scenario was further complicated by findings from Cox models, showing that having RAL trough concentrations below the assay detection limit immediately before or at one or more previous visit was also highly significantly associated with increased hazard of virologic failure. So, which conclusions on the value of DRV and RAL therapeutic monitoring can be drawn from this study?

We and others [2,3] have previously shown that RAL trough concentrations are associated with large inter-individual variability and, most importantly, largely failed to correlate with RAL area under the time–concentration curve $(AUC)_{0-12}$, taken as the golden standard pharmacokinetic parameter for the quantification of daily
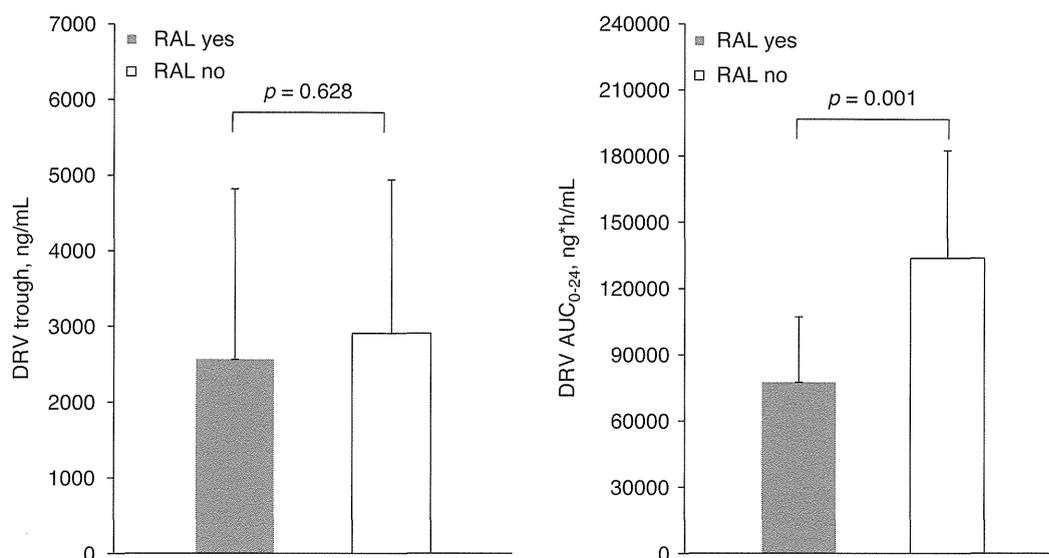
Fig. 1. Mean (±SD) darunavir (DRV) trough concentration (left panel) and area under the time–concentration curve (AUC)$_{0-24}$ (right panel) measured in patients given DRV with or without concomitant raltegravir (RAL) administration.

drug exposure. Moreover, recent studies have demonstrated that RAL has a resistance time on the integrase/DNA pre-integration complex that exceeds the half-life of the pre-integration complex in the cells [4,5]. Consequently, as the inhibition induced by RAL is functionally irreversible, no association between RAL pharmacokinetics and clinical outcome can be reasonably expected [6]. According to these findings, it is unlikely that RAL trough concentrations can per se directly affect response to therapy of patients enrolled in the ACTG trial. Therefore, other ways in which RAL could indirectly impact on patient outcome should be advocated.

A clear indication from the ACTG trial is that, according to sensitivity analyses, DRV concentrations were significantly lower in patients with virologic failure compared with those without virologic failure. Therefore, any factor able to affect DRV pharmacokinetics could theoretically impact on patient outcome. An intriguing hypothesis is that co-administration of RAL may lower plasma concentrations of DRV, as recently documented by Fabbiani et al. [7], ultimately resulting in suboptimal DRV exposure and poor response to combined RAL and DRV therapy. Unfortunately, no matched control patients given DRV/r at comparable dosage without RAL were available from the ACTG trial to compare DRV plasma trough concentrations in patients given or not given RAL. The authors have, however, rejected the hypothesis of a drug–drug interaction because the DRV trough concentrations measured in the ACTG trial were 'within the range previously reported in an intensive pharmacokinetic study of DRV 800/100 mg daily' [8]. Taiwo et al. [1] failed, however, to consider an important methodological drawback of their study. Particularly, they based their assumptions on the assessment of trough DRV

concentration as the solely pharmacokinetic drug parameter, which is not appropriate for the assessment of drug–drug interactions. Indeed, by performing detailed DRV pharmacokinetic evaluations in 25 HIV-infected patients [9], we have recently shown that co-administration of RAL did not impact on DRV trough levels, but was associated with highly significantly lower DRV AUC$_{0-24}$ compared with values measured in patients not given RAL (Fig. 1).

According to these findings, a potential pharmacokinetic drug–drug interaction between RAL and DRV ultimately affecting the results of the ACTG A5262 trial cannot be ruled out. This potential interaction should be investigated further and taken into account when DRV with RAL-based HAART regimens are implemented in the setting of HIV.

Acknowledgements

Conflicts of interest

Cristina Gervasoni[a] *and Dario Cattaneo*[b], [a]*Third Division of Infectious Diseases, and* [b]*Unit of Clinical Pharmacology, Luigi Sacco University Hospital, Università di Milano, Milan, Italy.*

Correspondence to Cristina Gervasoni, MD, Third Division of Infectious Diseases, Luigi Sacco University Hospital, via GB Grassi 74, 20157 Milan, Italy.
E-mail: cristina.gervasoni@unimi.it

References

1. Taiwo B, Zheng L, Gallien S, Matining RM, Kuritzkes DR, Wilson CC, et al. **Efficacy of a nucleoside-sparing regimen of darunavir/ritonavir plus raltegravir in treatment-naive HIV-1-infected patients (ACTG A5262)**. AIDS 2011; 25:2113–2122.
2. Cattaneo D, Ripamonti D, Gervasoni C, Landonio S, Meraviglia P, Baldelli S, et al. **Limited sampling strategies for the estimation of raltegravir daily exposure in HIV-infected patients**. J Clin Pharmacol 2011 [Epub ahead of print].
3. Burger D, Colbers EPH, van Luin M, Koopmans PP. **AUC0-3 h of raltegravir is correlated to AUC0-12 h: a novel approach for therapeutic drug monitoring of raltegravir** [abstract 41]. In: 11th International Workshop on Clinical Pharmacology of HIV Therapy; 7–9 April 2010; Sorrento, Italy.
4. Grobler JA, McKenna PM, Ly S. **Functionally irreversible inhibition of integration by slowly dissociating strand transfer inhibitor** [abstract O-10]. In: 10th International Workshop on Clinical Pharmacology of HIV Therapy; 15–17 April 2009; Amsterdam, the Netherlands.
5. McSharry J, Weng Q, Zager K, Soldani K, Kulawy R, Drusano G. **Pharmacodynamics of raltegravir, an HIV integrase inhibitor in an in vitro hollow fiber infection model system** [abstract A-960]. In: ICAAC/IDSA; October 2008; Washington, District of Columbia, USA.
6. Brainard DM, Wenning LA, Stone JA, Wagner JA, Iwamoto M. **Clinical pharmacology profile of raltegravir, an HIV-1 integrase strand transfer inhibitor**. J Clin Pharmacol 2011; 51:1376–1402.
7. Fabbiani M, Di Giambenedetto S, Ragazzoni E, D'Ettorre G, Parruti G, Prosperi M, et al. **Darunavir/ritonavir and raltegravir coadministered in routine clinical practice: potential role for an unexpected drug interaction**. Pharmacol Res 2011; 63:249–253.
8. Boffito M, Moyle M, Hill A, Sekar V, Lefebvre E, De Pauw M. **The pharmacokinetic profile of darunavir with low-dose ritonavir (DRV/r) in various multiple-dose regimens over 120 h** [abstract P-31]. In: 9th International Workshop on Clinical Pharmacology of HIV Therapy; 7–9 April 2008; New Orleans, Louisiana, USA.
9. Cattaneo D, Gervasoni C, Cozzi V, Baldelli S, Fucile S, Meraviglia P, et al. **Influence of raltegravir on the pharmacokinetics of darunavir in HIV-1-infected patients**. Pharmacol Res 2011 [Epub ahead of print].

Epstein–Barr virus associated colitis in an HIV-infected patient

Epstein–Barr virus (EBV)-associated lymphoma of the gastrointestinal tract is common in HIV-infected patients [1]. However, EBV involvement of the gastrointestinal tract without frank lymphoma is rare [2]. Although a few cases of EBV-associated colitis in both immunocompetent and immunocompromised patients have been reported [2,3], to our knowledge, EBV-associated colitis in HIV-infected patients has never been reported. We report a case of EBV-associated colitis with HIV infection successfully treated by combination antiretroviral therapy (cART).

A 40-year-old homosexual man who had had diarrhea for a few years developed bloody diarrhea. After persistence of the symptom for 2 months, he sought medical advice at a local hospital. He was diagnosed with HIV infection and referred to our hospital for further examination. On admission, the patient was alert with body temperature of 37.6°C. Physical examination showed oral candidiasis but no peripheral lymphadenopathy or abdominal tenderness. Laboratory tests at admission showed low CD4$^+$ cell count (84 cells/µl), anemia (hemoglobin 10.6 g/dl), low serum albumin (2.1 g/dl), and elevated C-reactive protein (3.45 mg/dl). Colonoscopy showed diffuse edematous mucosa with deep ulcers in the rectum, sigmoid colon, and descending colon (Fig. 1a), suggestive of either cytomegalovirus (CMV) colitis or amebic colitis. Based on the clinical suspicion, we initiated empirical treatment of gancicrovir and metronidazole. However, the results for amebic colitis such as stool microscopy, serum antiamebic antibody, and trophozoites in colonic biopsy specimens were all negative. Furthermore, histopathol-

ogy revealed no inclusion bodies and negative immunological staining for CMV. Then, we suspected inflammatory bowel disease (IBD), and mesalazine 4 g/day was initiated on day 7. Since there was no sign of other opportunistic infections, cART of raltegravir and emtricitabine/tenofovir was initiated on day 13. However, the bloody diarrhea persisted and a repeat colonoscopy was performed on day 19 to investigate the cause. The edematous mucosa and deep ulcers were still observed on colonoscopy. To identify infectious agents, a polymerase chain reaction (PCR) assay for EBV in the biopsy sample was performed, which showed 9000 copies/ml. Histopathological examination showed dense lymphoplasmacytic infiltration and mild neutrophil infiltration (Fig. 1c). In-situ hybridization (ISH) for EBV-encoded small RNA-1 (EBER-1) showed some positive cells (Fig. 1d). Based on these tests, the final diagnosis was established as EBV-associated colitis. The treatment plan included continuation of cART and withdrawal of mesalazine since IBD was considered unlikely. The symptom of bloody diarrhea gradually improved and disappeared by cART alone. At 3 months, the CD4 cell count had increased to 190/µl and the third colonoscopy showed significant improvement (Fig. 1b). PCR for EBV DNA in the biopsy sample showed a decrease to 80 copies/ml, and ISH showed no EBER-1-positive cells.

Although EBV-associated lymphoma of the gastrointestinal tract is common, EBV-associated colitis is very rare. To our knowledge, this is the first study demonstrating EBV-associated colitis in an HIV-infected patient. In
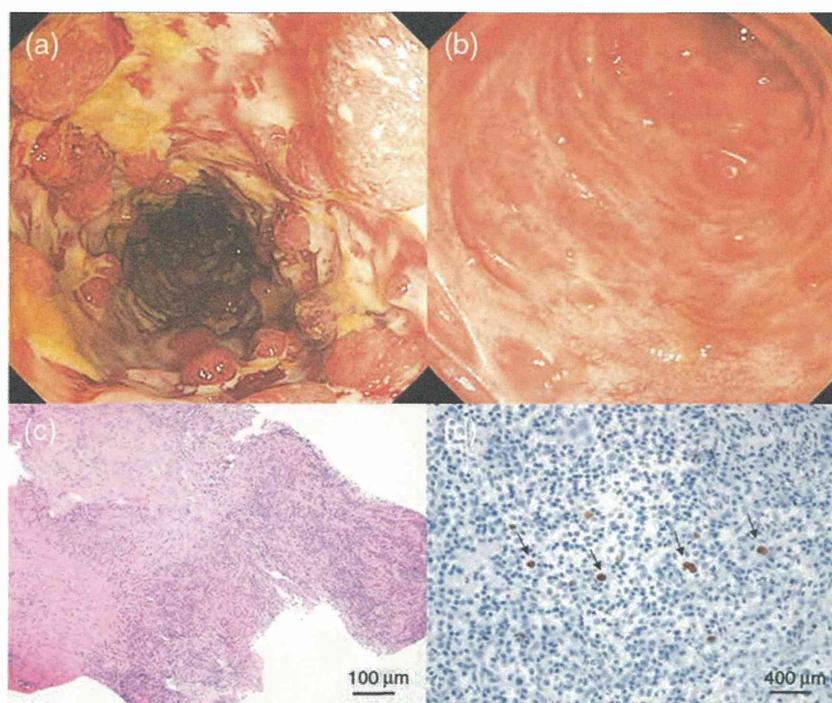
Fig. 1. **Endoscopic and microscopic findings.** Colonoscopic findings (a) on admission and (b) on 105th day of antiretroviral therapy. (c) Histopathological examination showing granulation tissue in ulcer floor (hematoxylin-eosin staining). (d) Epstein–Barr virus-encoded small RNA-1 in-situ hybridization demonstrated the presence of positive cells (black arrow).

addition, the significant improvement was achieved by cART alone. Several cases of EBV-associated colitis have been reported previously in immunocompromised patients, such as post-transplant patients and patients with IBD treated with immunosuppressants [2,3]. For this reason, EBV reactivation due to impaired immunity is considered to be a major causative factor of EBV-associated colitis.

In this case, EBV-associated colitis was diagnosed by the presence of EBV DNA and EBER-1-positive cells in the biopsy sample, and the improvement of colonoscopic findings associated with a decrease in EBV DNA. Because colonic appearance is grossly indistinguishable from that of CMV colitis, other forms of infectious colitis and IBD, positive EBV DNA and EBER-1 in the colonic specimens are important findings for establishing the correct diagnosis. In the case of delayed recognition of EBV colitis, treatment for IBD with corticosteroids can lead to unfavorable outcome [4]. Thus, EBV-associated colitis should be considered in HIV-infected patients, especially those with low $CD4^+$ cell counts, who present with colitis of unclear cause.

Because there is no established treatment for EBV infection, we treated this case with cART alone without specific treatment for EBV [5]. The loss of CMV viremia by cART in the absence of specific anti-CMV therapy has been reported previously [6]; therefore, it is likely that the suppression of EBV was also achieved by cART alone.

Because most reported cases of EBV colitis occurred from EBV reactivation due to impaired immunity, it is rational that restoration of the immune system by cART allowed the suppression of EBV activation and resulted in the resolution of colitis.

In conclusion, cART was effective against EBV-associated colitis. Clinicians should consider EBV infection in HIV-infected patients who present with colitis of unclear cause.

Acknowledgements

Yohei Hamada[a], Naoyoshi Nagata[b], Haruhito Honda[a], Naoki Asayama[b], Katsuji Teruya[a], Toru Igari[c], Yoshimi Kikuchi[a] and Shinichi Oka[a], [a]AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, [b]Department of Gastroenterology and Hepatology, and [c]Department of Pathology, Division of Clinical Laboratory, National Center for Global Health and Medicine, Tokyo, Japan.

Correspondence to Naoyoshi Nagata, MD, Department of Gastroenterology and Hepatology, National Center for Global Health and Medicine, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162-8655, Japan.
Tel: +81 3 3202 7181; fax: +81 3 3208 4244;
e-mail: nnagata_ncgm@yahoo.co.jp

References

1. Carbone A, Cesarman E, Spina M, Gloghini A, Schulz TF. **HIV-associated lymphomas and gamma-herpesviruses.** *Blood* 2009; **113**:1213–1224.

2. Karlitz JJ, Li ST, Holman RP, Rice MC. **EBV-associated colitis mimicking IBD in an immunocompetent individual.** *Nat Rev Gastroenterol Hepatol* 2011; **8**:50–54.

3. Tashiro Y, Goto M, Takemoto Y, Sato E, Shirahama H, Utsunomiya A, *et al*. **Epstein-Barr virus-associated enteritis with multiple ulcers after stem cell transplantation: first histologically confirmed case.** *Pathol Int* 2006; **56**:530–537.

4. Kobayashi CI, Yamamoto G, Hayashi A, Ota S, Imai Y, Fukayama M, *et al*. **Fatal amebic colitis after high-dose dexamethasone therapy for newly diagnosed multiple myeloma.** *Ann Hematol* 2011; **90**:225–226.

5. Torre D, Tambini R. **Acyclovir for treatment of infectious mononucleosis: a meta-analysis.** *Scand J Infect Dis* 1999; **31**:543–547.

6. Deayton J, Mocroft A, Wilson P, Emery VC, Johnson MA, Griffiths PD. **Loss of cytomegalovirus (CMV) viraemia following highly active antiretroviral therapy in the absence of specific anti-CMV therapy.** *AIDS* 1999; **13**:1203–1206.

Selection and Accumulation of an HIV-1 Escape Mutant by Three Types of HIV-1-Specific Cytotoxic T Lymphocytes Recognizing Wild-Type and/or Escape Mutant Epitopes

Tomohiro Akahoshi,[a] Takayuki Chikata,[a] Yoshiko Tamura,[a] Hiroyuki Gatanaga,[a,b] Shinichi Oka,[a,b] and Masafumi Takiguchi[a]

Center for AIDS Research, Kumamoto University, Kumamoto, Japan,[a] and AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan[b]

It is known that cytotoxic T lymphocytes (CTLs) recognizing HIV-1 escape mutants are elicited in HIV-1-infected individuals, but their role in the control of HIV-1 replication remains unclear. We investigated the antiviral ability of CTLs recognizing the HLA-A*24:02-restricted Gag28 -36 (KYKLKHIVW) epitope and/or its escape mutant (KYRLKHIVW) elicited in the early and chronic phases of the infection. Wild-type (WT)-epitope-specific CTLs, as well as cross-reactive CTLs recognizing both WT and K30R (3R) epitopes, which were predominantly elicited at early and/or chronic phases in HLA-A*24:02⁺ individuals infected with the WT virus, suppressed the replication of the WT virus but failed to suppress that of the 3R virus, indicating that the 3R virus was selected by these 2 types of CTLs. On the other hand, cross-reactive and 3R-specific CTLs, which were elicited in those infected with the 3R virus, did not suppress the replication of either WT or 3R virus, indicating that these CTLs did not contribute to the control of 3R virus replication. High accumulation of the 3R mutation was found in a Japanese population recently recruited. The selection and accumulation of this 3R mutation resulted from the antiviral ability of these Gag28-specific CTLs and high prevalence of HLA-A*24:02 in a Japanese population. The present study highlighted the mechanisms for the roles of cross-reactive and mutant-epitope-specific CTLs, as well as high accumulation of escape mutants, in an HIV-1-infected population.

Human immunodeficiency virus type 1 (HIV-1)-specific cytotoxic T lymphocytes (CTLs) play an important role in the control of HIV-1 during the acute and chronic phases of an HIV-1 infection (22, 40). However, HIV-1-specific CTLs cannot completely eliminate HIV-1-infected cells, because HIV-1 escapes from CTL-mediated immune pressure by various mechanisms, such as selection of escape mutations, Nef-mediated HLA class I downregulation, and skewed maturation of memory HIV-specific CD8⁺ T lymphocytes (5, 8, 9). The most documented escape mechanism is acquisition of amino acid mutations within the CTL epitope and/or its flanking regions. These mutations lead to reduced ability of peptide to bind to HLA class I molecules, impaired T cell receptor (TCR) recognition, and defective epitope generation (21, 31). These escape mechanisms are involved in impaired activities of HIV-1-specific CTLs to kill target cells infected with escape mutant virus and to suppress HIV-1 replication, contributing to the selection of escape mutant viruses (5, 10, 13, 20, 29, 35, 41).

There is growing evidence that escape mutations selected by HLA class I-restricted CTLs accumulate at the population level (7, 28, 36). The accumulation of escape mutants may affect the clinical outcomes for HIV-1-infected individuals (11, 37, 38). On the other hand, it is known that CTLs recognizing escape mutants are elicited after the emergence of the escape mutant selected by wild-type (WT) epitope-specific CTLs (2, 4, 12, 15, 33, 39). The escape mutant-specific CTLs were also elicited in new hosts carrying the same restricted HLA allele when they were infected with the mutant (15). Several studies showed that CTLs cross-recognizing the WT and its escape mutant epitopes are elicited before or after the emergence of the escape mutant in the same hosts (18, 25, 26, 33, 34). However, the antiviral abilities of these cross-reactive CTLs remain unknown, since the recognition of cross-reactive CTLs for synthesized epitope peptides

was characterized by using the enzyme-linked immunosorbent spot assay (ELISPOT) or ⁵¹Cr cytotoxic assay in those studies. We previously showed that HLA-A*24:02-restricted Nef 138-specific CTLs recognizing an escape mutant had weaker ability to suppress the replication of the mutant virus than that of the WT virus (15). However, it still remains unclear whether cross-reactive or escape mutant-specific CTLs contribute to the control of HIV-1, since the CTLs have not been analyzed in detail.

To clarify the abilities of cross-reactive and escape mutant-specific CTLs to recognize HIV-1-infected cells, we analyzed CTLs specific for HLA-A*24:02-restricted HIV-1 Gag28-36 (KYKLKH IVW; Gag28), which is the only immunodominant Gag epitope presented by this HLA class I allele (24). Since HLA-A*24:02 is found in approximately 70% of the Japanese population (42), the mutants of HLA-A*24:02-restricted epitopes may accumulate in HIV-1-infected Japanese individuals. We previously suggested that K30R (3R) in the Gag28 epitope is an escape mutation from HLA-A*24:02-restricted Gag28-specific CTLs (30) and that CTLs recognizing 3R are elicited in HIV-1-infected HLA-A*24:02⁺ individuals (46). From these studies, we hypothesized that cross-reactive CTLs recognizing WT and 3R mutant epitopes and/or 3R-specific CTLs are elicited in HLA-A*24:02⁺ HIV-1-infected individuals after the 3R mutant is selected and in new 3R virus-infected hosts carrying HLA-A*24:02. Here, we investigated the elicitation of Gag28-specific CTLs in 12 HLA-A*24:02⁺ HIV-1-