Correspondence

AIDS 2009, **23**:433–435

Successful genotype-tailored treatment with small-dose efavirenz

King and Aberg [1] recently published an excellent review of the clinical implications of population differences and genomic variations in efavirenz (EFV) treatment. They elegantly summarized the relationship between EFV concentration under standard dosage (600 mg once daily) and the genotype of cytochrome p450 2B6 (CYP2B6), a primary liver enzyme in EFV metabolism. They also highlighted the importance of CYP2B6 516 G>T SNP as a marker of individuals at risk of high EFV concentration and potential development of central nervous system (CNS) side-effects. However, it is desirable to discuss possible personalization of treatment by EFV dose modification.

As we described in our recent clinical study [2], we reduced EFV dosage in 12 patients with CYP2B6 516G>T polymorphism who were found to have extremely high EFV concentrations when treated with the standard dosage. The dosage was reduced from 600 to 400 mg in five individuals and to 200 mg in seven, and their HIV-1 load was successfully suppressed below detection limit (50 copies/ml) at these dosages. Interestingly, nine of the 12 suffered from chronic CNS-related symptoms at the standard dosage, but these improved in all nine by EFV dose reduction. An example of these patients is a 71-year-old man who reported having nightmares almost every night since starting EFV-containing antiretroviral therapy at 600 mg 3 years ago (Fig. 1). Plasma EFV concentrations were extremely high and analysis of the 516G>T SNP showed CYP2B6 516 genotype T/T. The EFV dosage was reduced to 400 mg. This resulted in a dramatic change in dream contents from nightmares to pleasant dreams. These changes occurred although the EFV concentration remained high at 400 mg. Therefore, we further reduced the dose to 200 mg. The second reduction resulted in complete disappearance of dreams. Although he missed the dreams, the EFV concentration decreased to within the target range at 200 mg. The EFV dose has been at 200 mg for more than 2 years, and the HIV-1 load remains under detection limit.

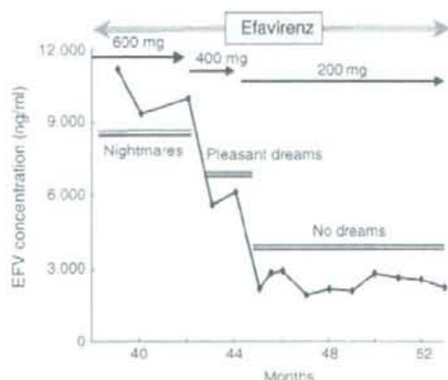

Fig. 1. Efavirenz dose reduction resulted in reduced efavirenz concentration and improved central nervous system related symptom. A CYP2B6 516T/T genotype holder reported having nightmares every night for 3 years, which disappeared after efavirenz (EFV) dose reduction.

Hasse *et al.* [3] also reported a patient with genotype CYP2B6 516T/T, who had chronic CNS symptoms and extremely high EFV concentration at 600 mg dose, but the symptoms resolved by reducing the EFV dose to 200 mg. Considered together, the above report and our study suggest that the quality of life of CYP2B6 516T/T genotype holders who suffer from CNS-related symptoms can be improved by reducing EFV dose from the standard to 400 or even 200 mg. In their review, King and Aberg [1] indicated that the cost remains an issue for identifying CYP2B6 516 genotype. However, one Japanese commercial laboratory has already developed a CYP2B6 516 genotype detection system based on the Invader assay [4], which costs only ¥8000 (~$75) per single test. Thus, the financial benefits of reducing EFV dosage should compensate for the cost of genotyping. Further large-scale studies are needed to discuss genotype-based tailored EFV treatment.

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References

1. King J, Aberg JA. **Clinical impact of patient population differences and genomic variation in efavirenz therapy.** *AIDS* 2008; **22**:1709–1717.

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2. Gatanaga H, Hayashida T, Tsuchiya K, Yoshino M, Kuwahara T, Tsukada H, et al. **Successful efavirenz dose reduction in HIV type 1-infected individuals with cytochrome P450 2B6 *6 and *26.** Clin Infect Dis 2007; **45**:1230–1237.
3. Hasse B, Gunthard HF, Bleiber G, Krause M. **Efavirenz intoxication due to slow hepatic metabolism.** Clin Infect Dis 2005; **40**:e22–e23.
4. Mein CA, Barratt BJ, Dunn MG, Siegmund T, Smith AN, Esposito L, et al. **Evaluation of single nucleotide polymorphism typing with invader on PCR amplicon and its automation.** Genome Res 2002; **10**:330–343.

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Successful use of darunavir, etravirine, enfuvirtide and tenofovir/emtricitabine in pregnant woman with multiclass HIV resistance

A 38-year-old HIV-1-positive African woman was presented to clinic at 6 weeks of a twin pregnancy. In the past, she had received nucleoside reverse transcripase inhibitors (zidovudine, lamivudine, tenofovir and didanosine), nonnucleoside reverse transcriptase inhibitors (efavirenz and nevirapine) and protease inhibitors (nelfinavir and lopinavir). Her switches in antiretroviral therapy (ART) had been because of virological failure related to poor adherence. Her treatment was tenofovir/emtricitabine (TDF/FTC) and boosted atazanavir. Her viral load was 4660 copies/ml with CD4 cell count of 471 cells/μl.

Therapeutic drug monitoring showed levels in excess for atazanavir. Sequencing of HIV polymerase revealed resistance mutations – reverse transcriptase: D67N, V118I/V, M184V, Y188L, L210W and T215Y; protease inhibitor: L10I, I13V, G16E, K20I, M36I, M46I, I47V, F53L, I54V, D60E, D63T, H69K, I84V and L89M. A phenotypic assay confirmed high-level resistance to almost all licensed antiretroviral drugs. Thus, there was a risk of mother-to-child transmission (MTCT) of multiclass-resistant HIV [multidrug-resistant HIV (MDR-HIV)]. After discussion by a multidisciplinary HIV team, at 25 weeks gestation, she was prescribed darunavir 600 mg twice daily (b.i.d) with ritonavir 100 mg b.i.d, etravirine 200 mg b.i.d (via compassionate release program) and enfuvirtide (T20) 90 mg subcutaneously b.i.d given under direct observation and TDF/FTC (245 mg/200 mg) one tablet q.d. as optimized background therapy. Four weeks later, her viral load was fully suppressed (HIV RNA <50 copies/ml) with CD4 cell count of 356 cells/μl. A pharmacokinetic study of T20, etravirine and darunavir showed maternal plasma levels that were above the expected therapeutic ranges (Table 1).

At 32 weeks gestation, she developed premature contractions and received oxytocin receptor antogonist (atosiban). After spontaneous rupture of membranes at 34 weeks gestation, a caesarean section was performed 3 h after onset of labour. She delivered a healthy baby boy and girl weighing 1.810 and 1.860 kg, respectively. Neither zidovudine nor nevirapine were administered during labour, but she received an extra dose of her current antiretroviral drugs 2.5 h before the caesarean section. At delivery, viral load remained undetectable, and her CD4 cell count was 451 cells/μl. The babies received a postnatal prophylactic antiretroviral drug regimen comprising T20 for 2 days, nevirapine for 1 week and didanosine for 2 weeks. Analysis of cord blood samples from both placentas showed undetectable levels of T20, whereas significant levels of darunavir, ritonavir and etravirine were found (Table 1). At 4 months of age, four HIV-1 DNA polymerase chain reaction tests performed on blood samples from each twin have been negative and no laboratory abnormalities noted.

Adverse events experienced by the mother were mild and included T20 injection site reactions, high fasting triglycerides and anaemia. She developed liver dysfunction at week 4 which peaked at week 8 of therapy (29 and 33 weeks gestation, respectively). All adverse events resolved spontaneously. Serological markers showed that she was hepatitis B immune with negative hepatitis B virus (HBV) DNA and negative for hepatitis A, C, cytomegalovirus (CMV), parvovirus, Q fever and rubella infections. Liver ultrasound was normal.

Use of ART in pregnancy significantly reduces MTCT of HIV [1]. This goal is more challenging in pregnant women with MDR-HIV. Newer antiretroviral drugs lack

Table 1. Levels of T20, ritonavir, darunavir and etravirine at week 4 (29 weeks of pregnancy) and at delivery (week 34) in cord blood samples.

					Cords sample (delivery)	
Twenty-nine weeks of pregnancy	Predose	1 h postdose	3 h postdose	6 h postdose	Twin 1	Twin 2
T20 (ng/ml)	3188	3766	4183	4313	Undetectable	Undetectable
Ritonavir (ng/ml)	199	365	188	212	25.7	123
Darunavir (ng/ml)	1960	2820	3940	3320	577	1020
Etravirine (ng/ml)	896	939	1110	1210	414	345

Levels of drugs were assayed by liquid chromatography-mass spectrometry/mass chromatography. Mean trough for wild-type virus in adults: T20: ranged from 2600 to 3400 ng/ml [12], darunavir: 3578 ng/ml (after 600/100 mg twice daily) [13] and etravirine: 297 ng/ml [14]. Ritonavir was used as booster.

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safety, tolerance and efficacy data in pregnancy. Neonatal MDR-HIV infection poses an even greater problem to treat, as there is no pharmacokinetic data for the newer drugs on infants.

Recent case reports highlighted the efficacy of T20 in preventing MTCT [2–4]. The lack of transplacental crossing renders this drug attractive for use in MDR-HIV-experienced pregnant women with anticipated little or no foetal toxicity [5]. Nevertheless, in one case report, T20-based regimen failed to prevent MTCT, despite an undetectable plasma viral load at delivery suggesting lack of genital tract penetration of such ART [6].

Unlike T20, placental crossing of darunavir and etravirine is not known. To our knowledge, ours is the first report to show transplacental transfer of darunavir and etravirine in human pregnancy. While raising concerns regarding possible foeto-toxicity, this finding also offers the prospect that these drugs could have prevented HIV infection in babies. Pregnant women have been excluded from all the major clinical trials conducted to date involving the three drugs [7–10]. In animal studies, however, no maternal foetal toxicity was observed for either darunavir or etravirine [11]. The extra dose of antiretroviral drugs given before the delivery may have influenced the cord blood levels, but caused no apparent neonatal toxicity. Clinicians should, where possible, give babies the same ART as the mother's regimen for prophylaxis, and we would advocate caesarean section as preferred mode of delivery in such cases.

In conclusion, we report successful prevention of MTCT in a case of MDR-HIV using a darunavir/etravirine-based regimen with evidence of placental transfer of both drugs.

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References

1. Townsend CL, Cortina-Borja M, Peckham CS, De Ruiter A, Lyall H, Tookey PA. **Low rates of mother-to-child transmission of HIV following effective pregnancy interventions in the United Kingdom and Ireland, 2000–2006.** *AIDS* 2008; **22**:973–981.
2. Meyohas MC, Lacombe K, Carbonne B, Morand-Joubert L, Girard PM. **Enfuvirtide prescription at the end of pregnancy to a multitreated HIV-infected woman with virological breakthrough.** *AIDS* 2004; **18**:1966–1968.
3. Brennan-Benson P, Pakianathan M, Rice P, Bonora S, Chakraborty R, Sharland M, Hay P. **Enfuvirtide prevents vertical transmission of multidrug-resistant HIV-1 in pregnancy but does not cross the placenta.** *AIDS* 2006; **20**:297–299.
4. Wensing AM, Boucher CA, van Kasteren M, van Dijken PJ, Geelen SP, Juttmann JR. **Prevention of mother-to-child transmission of multidrug resistant HIV-1 using maternal therapy with both enfuvirtide and tipranavir.** *AIDS* 2006; **20**:1465–1467.
5. Ceccaldi PF, Ferreira C, Gavard L, Gil S, Peytavin G, Mandelbrot L. **Placental transfer of enfuvirtide in the ex vivo human placenta perfusion model.** *Am J Obstet Gynecol* 2008; **198**: 433.e1–433.e2.
6. Cohan D, Feakins C, Wara D, Petru A, McNicholl I, Schillinger D, *et al.* **Perinatal transmission of multidrug-resistant HIV-1 despite viral suppression on an enfuvirtide-based treatment regimen.** *AIDS* 2005; **19**:989–990.
7. Clotet B, Bellos N, Molina JM, Cooper D, Goffard JC, Lazzarin A, *et al.* **Efficacy and safety of darunavir-ritonavir at week 48 in treatment-experienced patients with HIV-1 infection in POWER 1 and 2: a pooled subgroup analysis of data from two randomised trials.** *Lancet* 2007; **369**:1169–1178.
8. Madruga JV, Cahn P, Grinsztejn B, Haubrich R, Lalezari J, Mills A, *et al.* **Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-week results from a randomised, double-blind, placebo-controlled trial.** *Lancet* 2007; **370**:29–38.
9. Lazzarin A, Campbell T, Clotet B, Johnson M, Katlama C, Moll A, *et al.* **Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-2: 24-week results from a randomised, double-blind, placebo-controlled trial.** *Lancet* 2007; **370**:39–48.
10. Reynes J, Arastéh K, Clotet B, Cohen C, Cooper DA, Delfraissy JF, *et al.* **TORO: ninety-six-week virologic and immunologic response and safety evaluation of enfuvirtide with an optimized background of antiretrovirals.** *AIDS Patient Care STDS* 2007; **21**:533–543.
11. Raoof A, Lachau-Durand S, Verbeeck J, Bailey G, Martens M. **Etravirine has no effect on foetal development in rats and rabbits** [abstract]. In: *XVIIth International AIDS Conference*; 3–8 August 2008; Mexico City, Mexico. [Poster TUPE0013].
12. Roche Products Ltd. Fuzeon. Summary of product characteristics. http://emc.medicines.org.uk/emc/industry/default.asp?page=displaydoc.asp&documentid=12471.
13. Janssen-Cilag Ltd. Prezista. Summary of product characteristics. http://emc.medicines.org.uk/emc/industry/default.asp?page=displaydoc.asp&documentid=12471.
14. Tibotec Inc. Intelence (etravirine) Tablets US Prescribing Information. http://www.intelence-info.com/intelence/full-prescribing-info.html.

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OR, 6.2; 95% CI, 2.5–15.4). There was no increase in the risk of *T. vaginalis* infection among women who were infected with *T. vaginalis* during the immediately preceding interval (4.4%), compared with women who were not (3.9%). However, 13 (62%) of 21 new infections occurred in women who had been previously infected with *T. vaginalis*, and 11 (85%) of 13 had negative test results during the immediately preceding interval (figure 1).

Some of the women might have acquired infections during sexual contact that they did not report, and some might have had infections that were not detected at the baseline visit. However, many women were treated for infection, had negative test results, and then had positive test results again, which suggests that *T. vaginalis* was undetected by testing but still present for months after treatment. The possibility of long-term asymptomatic carriage is consistent with the age distribution of infected women; *T. vaginalis* is found more often in older women [8, 9]. This pattern is different from the pattern for bacterial sexually transmitted diseases but similar to that for incurable viral infections, such as herpes simplex virus type 2 [10]. Trials have suggested cure rates of >90%, but most have tested women once within a few weeks after treatment [11]. When women were tested again a few months after treatment, some of the previously cured women had infection detected again [11], and none of the studies continued testing women beyond a few months. Cultures might not detect infections if the concentration of *T. vaginalis* is low, which would be expected in asymptomatic infections [6, 12, 13]. Nucleic acid amplification tests may be better, but reports are inconsistent and the tests are not commercially available in the United States [14]. Similarly, self-obtained vaginal swab specimens occasionally miss infections, but the sensitivity of tests performed with self-obtained specimens has compared favorably with that of tests performed with clinician-obtained specimens [15].

Treatment failure could explain many of our findings, because 13 women had a documented preceding infection. However, our results were not simply attributable to treatment failure. Most of the women ($n = 11$) had an intervening negative test result before having a positive result during an interval when they reported not having sex. This suggests that, after treatment, *T. vaginalis* infection can become nondetectable for months and then reappear. Because these findings were unexpected and obtained with a small number of participants, additional studies are needed to confirm or refute these observations.

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References

1. Metcalf CA, Douglas JM Jr, Malotte CK, et al.; RESPECT-2 Study Group. Relative efficacy of prevention counseling with rapid and standard HIV testing: a randomized controlled trial (RESPECT-2). Sex Transm Dis 2005; 32:130–8.
2. Johnson RE, Newhall WJ, Papp JR, et al. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections—2002. MMWR Recomm Rep 2002; 51:1–38.
3. Weinhardt LS, Forsyth AD, Carey MP, Jaworski BC, Durant LE. Reliability and validity of self-report measures of HIV-related sexual behavior: progress since 1990 and recommendations for research and practice. Arch Sex Behav 1998; 27:155–80.
4. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines 2002. MMWR Recomm Rep 2002; 51:1–78.
5. Levi MH, Torres J, Pina C, Klein RS. Comparison of the InPouch TV culture system and Diamond's modified medium for detection of *Trichomonas vaginalis*. J Clin Microbiol 1997; 35:3308–10.
6. Caliendo AM, Jordan JA, Green AM, Ingersoll J, DiClemente RJ, Wingood GM. Real-time PCR improves detection of *Trichomonas vaginalis* infection compared with culture using self-collected vaginal swabs. Infect Dis Obstet Gynecol 2005; 13:145–50.
7. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986; 73:13–22.
8. Torok MR, Miller WC, Hobbs MM, et al. The association between *Trichomonas vaginalis* infection and level of vaginal lactobacilli, in nonpregnant women. J Infect Dis 2007; 196:1102–7.
9. Helms DJ, Mosure DJ, Metcalf CA, et al. Risk factors for prevalent and incident *Trichomonas vaginalis* among women participating in a randomized, controlled trial. Sex Transm Dis 2008; 35:484–8.
10. Centers for Disease Control and Prevention. Sexually transmitted disease surveillance, 2005. Atlanta, GA: US Department of Health and Human Services, 2006.
11. Forna F, Gulmezoglu AM. Interventions for treating trichomoniasis in women. Cochrane Database Syst Rev 2003; 2:CD000218.
12. Garber GE, Sibau L, Ma R, Procter EM, Shaw CE, Bowie WR. Cell culture compared with broth for detection of *Trichomonas vaginalis*. J Clin Microbiol 1987; 25:1275–9.
13. Radonjic IV, Dzamic AM, Mitrovic SM, et al. Diagnosis of *Trichomonas vaginalis* infection: the sensitivities and specificities of microscopy, culture and PCR assay. Eur J Obstet Gynecol Reprod Biol 2006; 126:116–20.
14. Schwebke JR, Burgess D. Trichomoniasis. Clin Microbiol Rev 2004; 17:794–803.
15. Schwebke JR, Morgan SC, Pinson GB. Validity of self-obtained vaginal specimens for diagnosis of trichomoniasis. J Clin Microbiol 1997; 35:1618–9.

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Detection of HIV Type 1 Load by the Roche Cobas TaqMan Assay in Patients with Viral Loads Previously Undetectable by the Roche Cobas Amplicor Monitor

To the Editor—In March 2008, the Roche Cobas TaqMan assay replaced the Roche Cobas Amplicor Monitor, version 1.5, for measuring plasma HIV type 1 (HIV-1) load in Japan. This has resulted

in the detection of an HIV-1 load >50 copies/mL in some of the patients whose HIV-1 load had been undetectable (<50 copies/mL) by the Amplicor Monitor over several years and for whom antiretroviral treatment regimens had not been changed [1, 2].

A total of 1387 HIV-1–infected patients visited our outpatient clinic from March through June 2008, and their HIV-1 load was measured by the TaqMan assay. Among these patients, 876 regularly visited the clinic (once every 1–3 months) and had an undetectable HIV-1 load by the Amplicor Monitor at the last visit. Surprisingly, the TaqMan assay detected an HIV-1 load >50 copies/mL in 253 (28.9%) of the 876 patients, although antiretroviral treatment had not been modified for these patients. Furthermore, another 22 patients (2.5%) were found to have an HIV-1 load >40 copies/mL with use of the TaqMan assay. The same assay also detected HIV-1 RNA at levels lower than the linear range of the assay (<40 copies/mL) in 128 (14.6%) of the 876 patients.

We analyzed the relationship between TaqMan detectability and time during which the HIV-1 load was undetectable by the Amplicor Monitor. This time was defined as the period from the first HIV-1 load undetectable by the Amplicor Monitor to the viral load first measured by the TaqMan assay, without any HIV-1 load rebound or blip detected during the period. Interestingly, among the patients who had a viral load undetectable by the Amplicor Monitor for <1 year, 43.7% had an HIV-1 load >50 copies/mL detected by the TaqMan assay; among the patients who had a viral load undetectable by the Amplicor Monitor for >4 years, 18.5% had an HIV-1 load >50 copies/mL detected by the TaqMan assay (figure 1). Conversely, 37.3% of patients who had a viral load undetectable by the Amplicor Monitor for <1 year had HIV-1 RNA undetectable by the TaqMan assay, and 70.2% of patients who had a viral load undetectable by Amplicor Monitor for >4 years had an HIV-1 load undetectable by the TaqMan assay. Thus, the proportion of patients who had an HIV-1 load >50 copies/mL was inversely correlated with the duration that the viral load was undetectable ($R^2 = 0.895$), and the proportion of patients with undetectable viral load was positively correlated with the duration that the viral load was undetectable ($R^2 = 0.979$). These findings indicate that the longer the effective treatment, the greater the number of patients with HIV-1 RNA undetectable by the TaqMan assay.

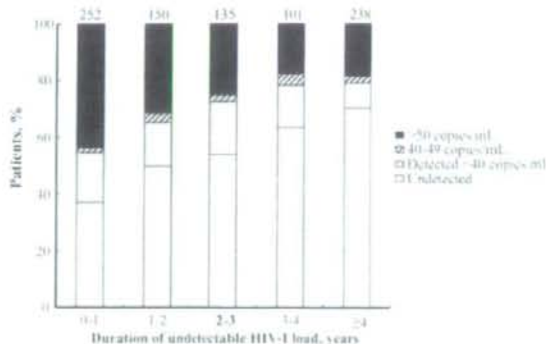

Figure 1. Results of the TaqMan assay and duration of undetectable HIV-1 load in 876 patients whose HIV-1 load was undetectable (<50 copies/mL) when the last Amplicor Monitor was performed. The number of patients is shown above each bar.

We observed significant discrepancy of HIV-1 detectability between the TaqMan assay and the Amplicor Monitor [3–5]. The TaqMan assay detected HIV-1 RNA in a significant percentage of treated patients with HIV-1 loads previously undetectable by the Amplicor Monitor; this is confusing to clinicians and patients and may be a critical problem in ongoing clinical trials of antiretroviral treatment. To determine the permissible range of detectable HIV-1 load during successful antiretroviral treatment, year-long clinical follow-up of treated patients is necessary. Our observation revealed that the detection rate of HIV-1 RNA with use of the TaqMan assay was inversely correlated with the previous duration of undetectable HIV-1 load, suggesting that long-term antiretroviral treatment can further suppress HIV-1 load even after it has decreased to below the detection limit of the Amplicor Monitor.

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References

1. Erali M, Hillyard DR. Evaluation of the ultrasensitive Roche Amplicor HIV-1 Monitor assay for quantitation of human immunodeficiency virus type 1 RNA. J Clin Microbiol **1999**; 37: 792–5.
2. Perrin L, Pawlotsky JM, Bouvier-Alias M, Sarrazin C, Zeuzem S, Colucci G. Multicenter performance evaluation of a new TaqMan PCR assay for monitoring human immunodeficiency virus RNA load. J Clin Microbiol **2006**; 44:4371–5.

High frequency and proliferation of CD4^{+}FOXP3^{+} Treg in HIV-1-infected patients with low CD4 counts

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The frequency of Treg is reported to be higher in patients with chronic HIV type 1 (HIV-1) infection and CD45RA^{+} Treg exist in normal adults. In this study, we found a lower absolute number (15 cells/μL) but a higher proportion (16.2%) of FOXP3^{+} cells (Treg) in the CD4^{+} population in treatment-naïve HIV-1 patients with low CD4 (<200 cells/μL) counts than in those with high CD4 counts (34 cells/μL and 9.3%) or healthy adults (48 cells/μL and 7.5%). In HIV-1 patients, CD45RA^{+}CCR7^{+}, CD45RA^{-}CCR7^{+}, and CD45RA^{-}CCR7^{-} subsets were identified in the Treg population, and the proportion of CD45RA^{-}CCR7^{-} Treg was higher (57.9%) in patients with low CD4 than high CD4 counts (38.3%). Treg were in a high proliferation state especially in patients with low CD4 counts. HIV viral load correlated positively with the Treg proliferation rate and the proportion of CD45RA^{-}CCR7^{-} Treg. Furthermore, the proliferation of Treg correlated positively with the CD45RA^{-}CCR7^{-} Treg proportion but negatively with Treg numbers. Successful antiretroviral therapy resulted in a limited increase in Treg numbers, but their frequency was reduced in 1–2 months due to a rapid rebound of FOXP3^{-} CD4^{+}cells. Our results suggest that HIV-activating Treg may be a reason for the high frequencies of Treg and CD45RA^{-}CCR7^{-} Treg in the peripheral blood of late-stage HIV-1-infected patients.

Key words: Cell proliferation · HIV · Immune regulation · Treg

Supporting Information available online

Introduction

HIV type 1 (HIV-1) infection is characterized by a progressive loss and dysfunction of CD4^{+} T cells [1, 2]. With regard to reduced T-cell functions, accumulating evidence suggests that the balance between the immune suppression function of natural Treg cells and the effector functions of other types of lymphoid cells influences the magnitude of immune reactions in various types of infections, *e.g.* those caused by *Leishmania major*, *Shistosoma mansonia*, and hepatitis C virus [3–7]. FOXP3 is not only a specific marker but also a critical lineage specification factor for Treg [8–11]. Treg are considered mainly as CD45RA^{-} cells. However, recent studies have shown that CD45RA^{+} cells also exist among immune-suppressing CD25^{+}CTLA4^{+}CD4^{+} T cells in adults [12, 13].

The local interaction between Treg and other T cells plays an important role in immune suppression and the local density of Treg determines the course of immune responses to infections [4, 7, 14]. Thus, Treg can be both detrimental and beneficial to the host in response to pathogens [5, 7]. For example, in HIV-infected patients, CD4^{+}CD25^{+} Treg have been reported to be proportionally increased, decreased, or highly increased in tonsils, their numbers to correlate with HIV viral load, and to exhibit suppression activity [15–23]. Furthermore, antiretroviral

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therapy (ART) has been reported to have either a negative or no influence on Treg or expression of FOXP3 [18, 23]. In HIV-1-infected individuals, immunodeficiency is often considered when the CD4 cell count falls below 200 cells/μL [1]. However, to our knowledge, there is controversy or little information about the absolute number, frequency, and status of homing markers of Treg in HIV-1-infected patients especially in those with low CD4 counts and late-stage AIDS-related diseases or not on ART [24, 25]. Little is known about the dynamic changes of Treg after ART has been introduced.

It is considered that the CCR7 molecule on T cells is an essential trafficking factor for T cells homing to lymphoid tissues as well as an important marker for defining differentiation stage of T cells with CD45RA molecule [26–28].

The present study was designed to investigate Treg in late-stage HIV-1-infected patients with CD4 count <200 cells/μL and the early impact of ART on Treg. We used the chemokine receptor CCR7 and CD45RA molecules to characterize distinct population of migratory Treg.

Results

High-frequency but low absolute numbers of Treg in HIV-1 patients with low CD4 counts

In this study, we enrolled 95 HIV-1-infected patients and 21 HIV-1-negative Japanese adults as our subjects. Because most AIDS-related diseases occur in HIV-1 patients when their CD4 count decreases to below 200 cells/μL, we classified the patients into two groups, a low CD4 group with a $CD4^+$ T cell count less than 200 cells/μL and a high CD4 group with a $CD4^+$ T cell count not less than 200 cells/μL, for some comparison analysis. Table 1 lists the demographic and clinical characteristics of HIV-1-infected patients and healthy HIV-1-negative controls.

Although FOXP3 expression is considered as the best and most specific marker of Treg, some studies have reported that CD127 and CD25 could distinguish Treg [29, 30]. Accordingly, we first compared the staining of FOXP3 with CD25 and CD127 using PBMC from HIV-1-positive individuals. As shown in Supporting Information Fig. 1A and B, $CD25^+CD127^-$ were a proportion of the CD4 cells. However, gating these cells as Treg seems difficult because of the smear staining of both CD25 and CD127. However, gating FOXP3 in CD4 cells was much easier because of the clear staining of FOXP3. Furthermore, we tested the correlation of the Treg by the two classification markers. Supporting Information Fig. 1C shows a good correlation between the proportion of $FOXP3^+$ and $CD25^+$ $CD127^-$ in CD4 cells in 18 HIV-1 patients. Therefore, in the present study, we considered the $FOXP3^+CD4^+$ cells as Treg, and called $FOXP3^-CD4^+$ cells as conventional $CD4^+$ T cells (Tcon).

In the next step, we investigated the frequency and absolute number of Treg in HIV-1-infected individuals without an ART history and compared them with those of healthy Japanese adults. Figure 1A and B shows FOPX3 expression in $CD4^+$ cells. As shown in Table 2, the proportion of Treg in CD4 cells was $16.2\pm2.6\%$ in HIV-1 patients with a low CD4 count and

Table 1. Demographic and clinical characteristics of subjects

	Group[a)]		
	A (low CD4)	B (high CD4)	H (healthy)
Characteristics			
Numbers	27	68	21
Age (years, range)	39 (21–65)	38 (21–67)	38 (21–60)
Gender (male:female)	27:0	16:1	3:4
CD4 count (cells/μL, SD)	102 (58)	383 (164)	650 (178)
LogVL (SD)	5 (0.6)	4.2 (0.7)	N/A
AIDS-related diseases[b)] (n, %)	23 (85)	11 (16)	N/A
Months of HIV^+ (range)[c)]	12.3 (0–97)	21 (0–124)	N/A
Numbers for tests			
Frequency and subsets of Treg[d)]	20	39	21
Ki67 staining *versus* FOXP3[e)]	11	24	5
CCR7FOXP3 *versus* CD25[f)]	3	16	
CD127CD25 *versus* FOXP3[g)]	6	12	

[a)] Low CD4: <200 cells/μL; high CD4: ≥ 200 cells/μL.
[b)] AIDS-related diseases included: candida, herpes simplex virus infection, tuberculosis, pneumocystis jirovici pneumonia, lymphoma (kaposis sarcoma), *etc.*
[c)] Months between the date of the first time of consulting the hospital and the date of blood collected.
[d)] Table 2 and Fig. 1.
[e)] Figure 2 and Supporting Information Fig. 2.
[f)] Figure 1C.
[g)] Supporting Information Fig. 1.

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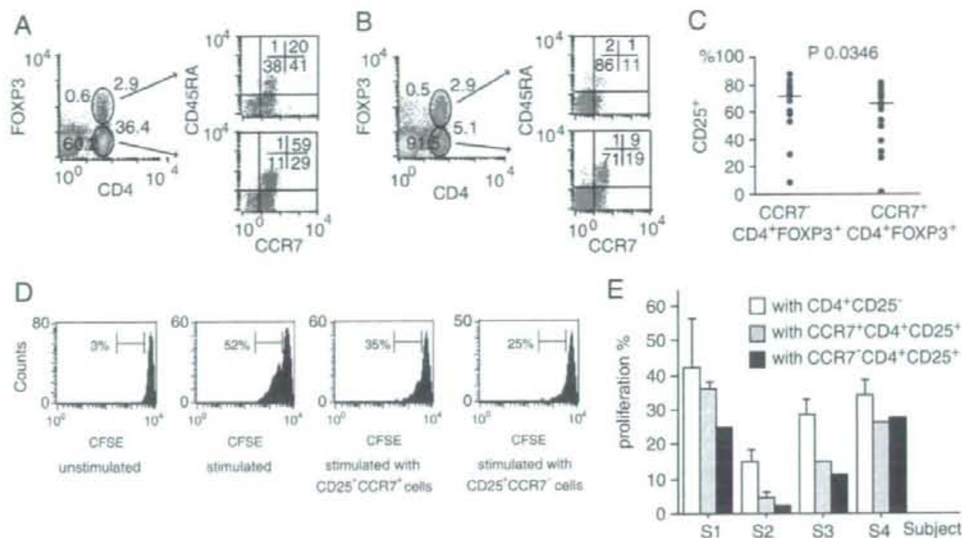

Figure 1. Subsets of Treg in healthy adults and HIV-1-infected patients. (A) Staining of a healthy adult. (B) Staining of an HIV-1-infected patient with low CD4 count. FOXP3 was mainly found in CD4$^+$ T cells both in healthy adults and HIV-1 patients. Treg (FOXP3$^+$CD4$^+$) cells could be subdivided into CD45RA$^+$CCR7$^+$, CD45RA$^-$CCR7$^+$, and CD45RA$^-$CCR7$^-$ subsets, similar to Tcon (FOXP3$^-$CD4$^+$, conventional CD4 cells). (C) In HIV-1 patients, the proportion of CD25$^+$ among CCR7$^-$ Treg was higher than that among CCR7$^+$ Treg ($p<0.05$, $n=19$). (D) A representative proliferation of CD4$^+$CD25$^-$ responder cells cultured with CCR7$^-$CD25$^+$CD4$^+$, CCR7$^+$CD25$^+$CD4$^+$ cells, or unlabeled CD25$^-$CD4$^+$ cells stimulated by anti-CD3 mAb with autologous APC (the data are derived from healthy control). (E) CCR7$^+$ and CCR7$^-$ Treg suppression of responder cells in four subjects. S1–S3: healthy subjects, S4: HIV-1-positive patient (the error bars show duplicate or triplicate tests). Horizontal bars represent median values and p value represents comparison result from Wilcoxon-signed rank test.

$9.3 \pm 0.5\%$ in patients with a high CD4 count. The absolute counts of Treg in low CD4 and high CD4 groups were 15 ± 3 and 34 ± 2 cells/μL, respectively. In healthy adults, the mean CD4 count was 650 cells/μL, and the frequency of Treg among CD4$^+$ cells was $7.5 \pm 0.5\%$ with a mean absolute number of 48 ± 4 cells/μL. Therefore, HIV-1 patients with low CD4 counts had a lower absolute count but a significantly higher frequency of Treg than HIV patients with high CD4 and healthy controls.

High proportion of CD45RA$^-$CCR7$^-$ Treg in HIV-1 patients with low CD4

Considering the distinct homing potentials and effector functions, CD4 T cells could be subdivided into three subsets, namely naïve (CD45RA$^+$CCR7$^+$), central memory (CD45RA$^-$CCR7$^+$), and effector memory (CD45RA$^-$CCR7$^-$) cells, based on their surface marker and cytokine secretion [26]. Given that local interaction of Treg and Tcon plays an important role in immune suppression and the local number and/or density of Treg reflects immune suppression, we next investigated whether Treg have the same characteristic phenotype as Tcon. Figure 1A shows that Treg could be divided into three subsets, similar to Tcon, based on CD45RA and CCR7 staining in healthy controls. Interestingly, the proportion of each subset of Treg was different compared with the respective subsets of Tcon (Table 2). In healthy adults, the proportion of CD45RA$^-$CCR7$^-$ Treg ($39.7 \pm 2\%$) was higher than CD45RA$^-$CCR7$^-$ Tcon cells ($15.6 \pm 1.2\%$), but the proportion of CD45RA$^+$CCR7$^+$ Treg ($19.3 \pm 1.6\%$) was lower than CD45RA$^+$CCR7$^+$ Tcon cells ($45.8 \pm 2.4\%$).

In HIV-1-infected patients, the staining patterns of intracellular FOXP3 and surface CD4, CD45RA, and CCR7 were similar to those in healthy controls (Fig. 1A and B). Figure 1B shows a high proportion of CD45RA$^-$CCR7$^-$ Treg in a representative patient with a low CD4 count. As shown in Table 2, the proportion of CD45RA$^-$CCR7$^-$ Treg in the low CD4 group ($57.9 \pm 4.2\%$) was significantly higher than in the high CD4 ($38.3 \pm 1.8\%$) or control groups ($39.7 \pm 2\%$). In contrast, the proportion of CD45RA$^-$CCR7$^+$ Treg in patients with low CD4 counts was significantly lower than in those with high CD4 counts and the control groups. In all subject groups, the proportions of CD45RA$^-$ cells in Treg were higher than in Tcon. Moreover, we found that in HIV-1-infected patients, the proportion of CD25$^+$ in CCR7$^-$ Treg ($64 \pm 19\%$) was higher than in CCR7$^+$ Treg ($58.8 \pm 21\%$, Fig. 1C).

CD45RA$^+$ Treg have been reported to show suppressive function [12]. Based on the finding of a high proportion of CCR7$^-$ Treg in patients with a low CD4 count (Table 2), and considering that CCR7$^+$ cells tend to home to lymphoid tissues whereas CCR7$^-$ cells tend to move to peripheral tissues, we next investigated whether there is any difference in the suppressive activity between CCR7$^+$ and CCR7$^-$ Treg. The results showed

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Table 2. Comparison of Treg and Tcon in healthy persons and HIV-1-infected patients[a]

	Healthy (H)	HIV-1(+)ART(−)		p value		
		CD4<200 (A)	CD4≥200 (B)	A *versus* B	A *versus* H	B *versus* H
Number of subjects	21	20	39			
Lymphocytes (cells/μL)	1718 (381)	1028 (447)	1661 (579)	<0.0001	<0.0001	NS
CD4 (cells/μL)	650 (178)	108 (58)	395 (195)	<0.0001	<0.0001	<0.0001
CD4 (%)	38.4 (8.6)	11.4 (7.6)	20.5 (8.5)	0.0001	<0.0001	<0.0001
Treg (cells/μL)	48 (16)	15 (11)	34 (14)	<0.0001	<0.0001	0.0008
Treg (%)	7.5 (2.4)	16.2 (11.8)	9.3 (3.4)	0.0137	0.0004	0.0464
Treg (%)						
$CCR7^+$	57	40.1	59.6	0.0001	0.0029	NS
$CD45RA^+CCR7^+$	19.3	13.4	21.1	0.0109	0.0504	NS
$CD45RA^-CCR7^-$	39.7	57.9	38.3	0.0001	0.0006	NS
$CD45RA^-CCR7^+$	37.7	26.7	38.5	0.0005	0.0057	NS
$CD45RA^-$	77.4	84.6	76.8	0.0131	0.0419	NS
Tcon (%)						
$CCR7^+$	81.3	55.8	74.8	0.0178	0.0035	NS
$CD45RA^+CCR7^+$	45.8	31.9	41.1	NS	0.0217	NS
$CD45RA^-CCR7^-$	15.6	36.8	22.1	0.0283	0.0035	0.04
$CD45RA^-CCR7^+$	35.5	23.9	33.7	0.0048	0.0045	NS
$CD45RA^-$	51.1	60.7	55.8	NS	NS	NS
p Value (Treg *versus* Tcon)						
$CCR7^+$	<0.0001	0.0187	<0.0001			
$CD45RA^+CCR7^+$	<0.0001	0.0001	<0.0001			
$CD45RA^-CCR7^-$	<0.0001	0.0004	<0.0001			
$CD45RA^-CCR7^+$	NS	NS	0.005			
$CD45RA^-$	<0.0001	<0.0001	<0.0001			

[a] Data are means (SD). NS: not significant. CD4<200, CD4≥200: 200 cells/μL. Mann–Whitney U-test was used for comparison between groups (A *versus* B, A *versus* H, B *versus* H). Wilcoxon-signed rank test was used for comparison in group (Treg *versus* Tcon).

that both $CCR7^+$ and $CCR7^-$ $CD25^+CD4^+$ cells suppressed the proliferation of responder cells (Fig. 1D). The suppressive activity was observed in three healthy controls and one HIV-1 patient (Fig. 1E), although no difference was found in the suppression function between the $CCR7^+$ and $CCR7^-$ Treg.

The above results demonstrated the existence of $CD45RA^+$ $CCR7^+$, $CD45RA^-CCR7^+$, and $CD45RA^-CCR7^-$ Treg subsets, similar to Tcon. The proportion of $CCR7^+$ Treg was lower than $CCR7^+$ Tcon cells in both healthy controls and HIV-1 patients. However, the proportion of $CD45RA^-CCR7^-$ Treg was higher than $CD45RA^-CCR7^-$ Tcon, particularly in patients with low CD4 count.

High proliferation of Treg correlates with HIV-1 viral load

Immune cells are activated in HIV-infected patients and such activation is linked to CD4 cell depletion [31]. To determine the mechanism of the high frequency of Treg and $CD45RA^-CCR7^-$ Treg in advanced HIV patients, we stained CD4 cells for the proliferation markers Ki67 in 24 patients (including 11 patients with low CD4 counts and 13 patients with high CD4 counts) and five healthy controls. Figure 2A shows that there was no difference between gating the Ki67 in Treg and Tcon in a healthy control and an HIV-1-infected person. As shown in Fig. 2, the proportions of Ki67-stained cells among Treg in low CD4, high CD4, and control groups (41.7, 24.5, and 22.3%, respectively) were higher than those in Tcon cells (18.1, 11.8, and 7.4%, respectively) (Fig. 2B). The expression of Ki67 in both Treg and Tcon cells was higher in patients with low CD4 counts than in those with high CD4 counts and healthy controls. Furthermore, in the 24 HIV-1-infected patients assessed for Ki67, HIV-1 viral load showed a positive correlation with the frequency of Ki67 in Treg and the proportion of $CD45RA^-CCR7^-$ in Treg. However, the CD4 count showed a negative correlation with the frequency of Ki67 in Treg (Fig. 2C). Moreover, the frequency of Ki67 in Treg correlated negatively with the Treg count and the proportion of $CD45RA^+CCR7^+$ in Treg, but positively with the proportion of $CD45RA^-CCR7^-$ in Treg (Fig. 2D). The same correlation was also observed in Tcon cells (Supporting Information Fig. 2).

ART reduces the frequency of Treg

In HIV-1-infected patients, ART can effectively reduce the HIV viral load and improve CD4 counts. In highly active ART-treated patients, a depleted or normalized Treg was observed in

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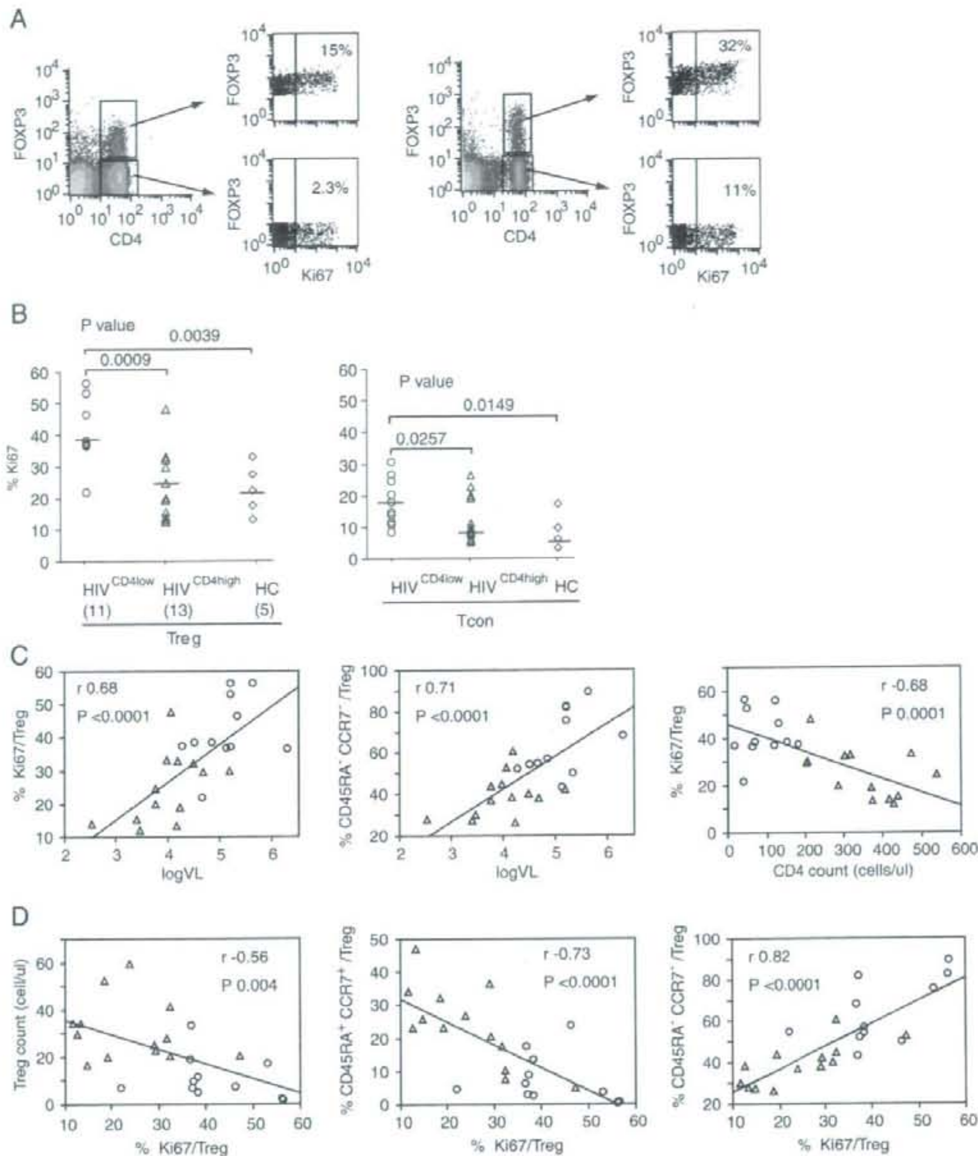

Figure 2. Ki67 staining and high proliferation rate of Treg is associated with viral load. (A) Gating of Ki67 in FOXP3$^+$ and FOXP3$^-$ CD4$^+$ cells in a healthy control (left panel) and an HIV-1-infected person (right panel). (B) Proportion of Ki67-positive Treg (left panel) is higher than that of Ki67-positive Tcon cells (right panel) in healthy controls (HC), HIV-1-infected patients with low CD4 count (HIV^{CD4low}) and HIV-1-infected patients with high CD4 count (HIV^{CD4high}) (numbers in parentheses represent the number of subjects tested). The percentages of Ki67-positive Treg and Tcon cells in the low CD4 group are higher than those in the high CD4 group and healthy control, respectively. (C) HIV-1 viral load shows a positive correlation with the percentage of Ki67 in Treg (left panel) and the proportion of CD45RA$^-$CCR7$^-$ Treg (middle panel). The CD4 count shows a negative correlation with the percentage of Ki67 in Treg (right panel). (D) The percentage of Ki67 in Treg shows correlation negatively with Treg count (left panel) and the proportion of CD45RA$^+$CCR7$^+$ Treg (middle panel) but positively with the proportion of CD45RA$^-$CCR7$^-$ Treg (right panel). Horizontal bars represent median values and p values represent results from Wilcoxon-signed rank test. Simple regression was used for correlation analysis.

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PBMC and mucosal tissue [23, 32]. To investigate the impact of ART on Treg, we checked the dynamic change in Treg, their proliferation state, and subsets in nine patients until 9 months after commencement of ART (Fig. 3). The plasma viral load decreased sharply soon after commencement of ART (Fig. 3A). Associated with the decrease in viral load was a rise in the CD4^{+} count especially in the first 2 months of ART. The CD4 count increased more than 100 cells/μL average in the first month (Fig. 3B). The absolute count of Treg increased in the first month but decreased to some extent thereafter (Fig. 3C); the frequency of Treg decreased rapidly to normal levels within 1–2 months of commencement of ART in all patients (Fig. 3D). On the other hand, the change in the proportion of Ki67 among Tcon and Treg showed a complex pattern. The proportion of Ki67 among Tcon cells increased in the first month of treatment and then decreased and fluctuated on a small scale thereafter (Fig. 3E). However, in the first 1–2 months of ART, the proportion of Ki67 among Treg decreased but maintained high levels until 9 months of ART (Fig. 3F). There was no significant change in each subset in both Treg and Tcon (Fig. 3G and H). However, the CD45RA^{-}CCR7^{-} subset still accounted for a high proportion, especially in Treg (Fig. 3G and H, the right panels). The detailed change of each item in each patient is shown in Supporting Information Fig. 3. These results suggest that after initiation of ART, the slow change in the absolute number of Treg and the rapid rebound of Tcon counts resulted in a rapid normalization of the frequency of Treg in HIV-1 patients.

Discussion

Regulation of the immune response is important in maintaining self-tolerance. However, in individuals with immunodeficiency, such as patients with HIV infection, severe immune suppression may contribute to progression of AIDS. Previous studies reported activation of the immune system in HIV-1-infected patients and indicated that human CD4^{+}CD25^{high}FOXP3^{+} Treg cells are derived through rapid turnover of memory populations *in vivo* [31, 33, 34].

In the present study, we found that untreated HIV-1-infected patients with low CD4 counts have a high frequency of Treg and CD45RA^{-}CCR7^{-} Treg. Cell proliferation was higher in Treg than Tcon cells, especially in HIV-1 patients with low CD4 counts. In these patients, both Tcon and Treg showed a high proliferation state, particularly about 40% Treg were Ki67-positive. Ndhlovu *et al.* [22] reported that FOXP3^{+}CD127^{lo} CD4^{+} T cells in PBMC showed a strong negative correlation with T-cell activation during the early chronic stage of HIV infection. In our study, we also found a negative correlation between the proliferating frequency of Treg and Treg absolute count. However, we found that the proliferation of Treg correlated positively with the proportion of CD45RA^{-}CCR7^{-} Treg. Furthermore, HIV viral load showed a positive correlation with both Treg proliferation and the proportion of CD45RA^{-}CCR7^{-} Treg. These results suggest that HIV infection may activate Treg and result in an increased proportion of CD45RA^{-}CCR7^{-} among Treg. On the other hand, Epple *et al.* [32] reported that the frequency and absolute counts of mucosal Treg were highly increased in untreated HIV patients. This finding may be considered another reason for our results because CCR7^{+} lymphocytes tend to home to lymph nodes and lymphoid tissues. Therefore, we consider that in HIV-infected patients, HIV could simultaneously activate the differentiation of Treg as well as stimulate CCR7^{+} Treg homing to lymph nodes and lymphoid tissues. These two effects of HIV on Treg result in the high frequency of Treg and a high proportion of CD45RA^{-}CCR7^{-} Treg in peripheral blood in patients with low CD4 counts.

ART has been a great success in controlling HIV replication and aiding the recovery of CD4 T cells. However, data about its impact on Treg, especially in detail, are rare. In the current study, we observed that with the rapid decrease in viral load was a robust rebound of Tcon 1–2 months after ART initiation; however, the number of Treg increased in some patients but was almost unchanged in others. The unbalanced change in Tcon and Treg resulted in the frequency of Treg decreasing precipitously to normal levels in the first 1–2 months of therapy. Although the viral load decreased to a very low level in a short period after ART introduction, the proliferative state of Tcon and Treg did not decrease significantly. On the contrary, both Tcon and Treg maintained a high proliferation level, especially Treg. Moreover, the three subsets, *i.e.* CD45RA^{+}CCR7^{+}, CD45RA^{-}CCR7^{+}, and CD45RA^{-}CCR7^{-} in Tcon and Treg did not show a robust change till 9 months. The results suggest that the recovery of phenotypes needs a much longer period, even if they can recover after ART.

Chase *et al.* [23] observed Treg depletion in highly active ART-treated HIV-1 patients but not in elite suppressors. Here, we did not observe depletion of Treg counts after ART introduction, but we indeed noticed a rapid normalization of the Treg frequency. As we know, to do the suppression assay *in vitro*, an appropriate ratio of Treg to responder cells is needed for observing significant suppression. Considering the suppressive function of both CCR7^{+} and CCR7^{-} Treg, we think that the high frequency of Treg, but not the low absolute number of Treg, provides a much better suppressive marker in treatment-naïve HIV-1 patients with low CD4 counts. On the other hand, ART may induce some improvement of the immune suppression because it could reduce the frequency of Treg.

In summary, our results of high frequencies of Treg and CD45RA^{-}CCR7^{-} Treg, which tend to migrate to non-lymphoid tissues, in untreated HIV-1 patients with low CD4 counts, emphasize the potential role of Treg in immune deficiency in late-stage HIV-1 infection. Furthermore, anti-HIV treatment could result in a rapid rebound of conventional T cells but not a robust improvement of Treg within 9 months after ART initiation. The different response of Treg and Tcon to ART leads to a rapid decrease in the frequency of Treg. Recently, immune reconstitution syndrome (IRS) is becoming an important problem in HIV treatment. Most IRS occurs in 1–3 months after commencement

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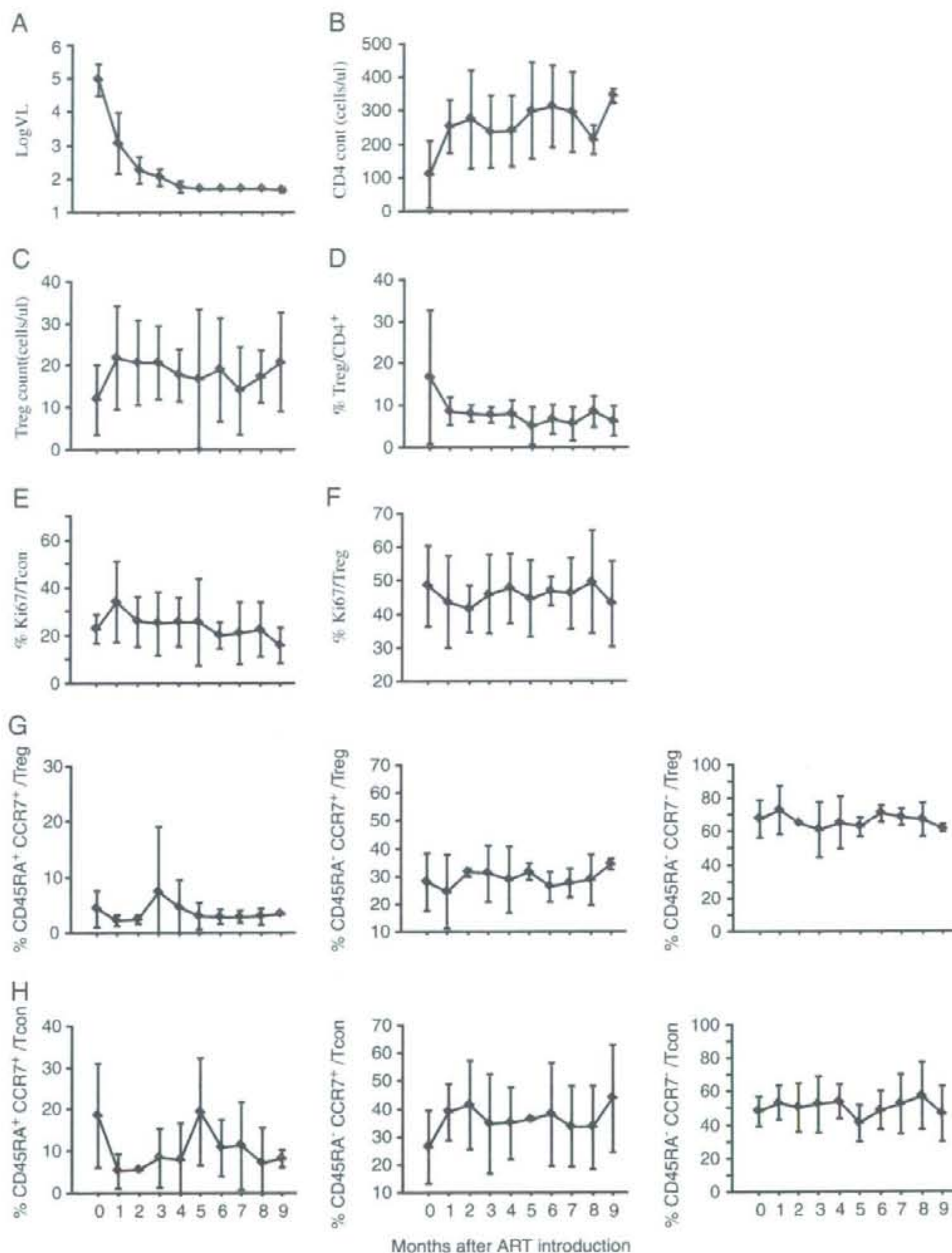

Figure 3. Serial changes in Treg and Tcon cells after commencement of ART. Commencement of ART resulted in rapid reduction in HIV viral load (A) and increase in $CD4^+$ cell count (B). Treatment caused increase in the absolute number of Treg in the first month, then fluctuated slightly thereafter (C), but resulted in a sharp decrease in their percentages in 1 month (D). The proportion of Ki67-positive Tcon increased in the first month but decreased in some extent thereafter (E), while the proportion of Ki67-positive Treg showed some change but still retained a high level at 9 months of commencement of ART (F). At 9 months after ART started, the recovery of the proportion of $CD45RA^+CCR7^+$ Treg (G, left panel) and Tcon (H, left panel) seems very slow, while the proportion of $CD45RA^-CCR7^+$ Treg (G, middle panel) and Tcon (H, middle panel) increased in some extent. However, the proportion of $CD45RA^-CCR7^-$ Treg (G, right panel) and $CD45RA^-CCR7^-$ Tcon (H, right panel) showed a small-scale change, but $CD45RA^-CCR7^-$ Treg maintained a high proportion till 9 months. (A–F) was from nine patients, while (G–H) was from six of them. Vertical bars represent mean $\pm$ 1SD.

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of ART. Thus, we suppose that the unbalanced improvement of conventional CD4 cells and Treg after commencement of ART might be a factor for IRS. However, this issue needs more investigation.

Materials and methods

Subjects

The subjects were 95 HIV-1-infected patients who have not received any ART and gave written consent before enrollment in this study at the AIDS Clinical Center, International Medical Center of Japan, Tokyo. Nine patients who started ART were followed up for investigation of the impact of ART on Treg. Twenty-one HIV-1-negative adults were recruited as healthy controls. The demographic and clinical characteristics of the subjects are listed in Table 1. HIV-1 viral load was quantified by AMPLICOR HIV-1 MONITOR Test (Roche Diagnostics).

Cell preparation

PBMC were prepared from blood samples collected into EDTA-containing tubes by Ficoll-paque gradient centrifugation. Ki67 staining and evaluation of the ART-treated patients were carried out using cryopreserved PBMC.

For suppression assay, $CD4^+$ cells were isolated from freshly prepared PBMC by using $CD4^+$ T-cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to instructions provided by the manufacturer. $CD4^+$ cells were separated by anti-CD25 mAb (PE) and anti-PE Multisort Kit (Miltenyi) into $CD25^-$ and $CD25^+$ cells. After microbeads release, $CD25^+$ cells were sorted into $CCR7^+$ and $CCR7^-$ cells by using anti-CCR7 mAb (FITC, mouse IgG2a, R&D Systems, Minneapolis, MN) and Rat Anti-Mouse IgG2a+b Microbeads (Miltenyi). The $CD4^+$ $CD25^-$ cells were labeled by 2 μM 5-6-CFSE as responder cells in the suppression assay. Unlabeled $CD4^+CD25^-$ cells were used as non-Treg for cell number control. PBMC that were depleted of $CD3^+$ cells by CD3 MicroBeads (Miltenyi) and irradiated with 3000 rad were used as APC.

Cell staining and flow cytometry

Freshly isolated PBMC were surface stained and also stained intracellularly for FOXP3 (PE/APC labeled, clone PCH101, eBioscience, San Diego, CA) and other markers. The stained cells were analyzed on Becton Dickinson FACSCalibur with CellQuest software (BD Bioscience, San Jose, CA). The monoclonal antibodies used in these staining procedures included anti-CCR7-FITC, anti-CD4-perCP, anti-CD25-PE, anti-CD45RA-APC/perCP, anti-Ki67-PE (BD PharMingen, San Diego, CA), and anti-CD127-FITC (eBioscience).

In vitro suppression assay

In a 96-well, round-bottom plate coated with anti-CD3 mAb (0.25–0.5 μg/mL), 5×10^4 CFSE-labeled $CD4^+CD25^-$ cells were seeded and followed by adding autologous APC (2.5×10^4). For testing Treg suppression, the same number of $CD4^+CD25^+$ $CCR7^+$ or $CCR7^-$ cells was added as regulatory cells. In control wells, the same number of unlabeled non-Treg $CD4^+CD25^-$ cells was added in order to adjust cell numbers in each well. After 3–4 days culture in an incubator at 37°C under 5% CO_2, the cells were harvested and analyzed on FACSCalibur. Live cells were gated and the dilution of CFSE was measured as proliferation of responder cells.

Statistical analysis

Data are expressed as mean ± SD. Differences between groups or stratified groups were examined for statistical significance using Mann–Whitney *U*-test and Wilcoxon-signed rank test. Simple linear regression was used for correlation analysis. All analyses were conducted using the StatView software (version 5.0). A p value of <0.05 was considered statistically significant.

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References

1 **Douek, D. C., Picker, L. J. and Koup, R. A.**, T cell dynamics in HIV-1 infection. *Annu. Rev. Immunol.* 2003. **21**: 265–304.

2 **Gandhi, R. T. and Walker, B. D.**, Immunologic control of HIV-1. *Annu. Rev. Med.* 2002. **53**: 149–172.

3 **Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M. and Toda, M.**, Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 1995. **155**: 1151–1164.

4 **Mills, K. H.**, Regulatory T cells: friend or foe in immunity to infection? *Nat. Rev. Immunol.* 2004. **4**: 841–855.

5 **Rouse, B. T., Sarangi, P. P. and Suvas, S.**, Regulatory T cells in virus infections. *Immunol. Rev.* 2006. **212**: 272–286.

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6 **Klenerman, P. and Hill, A.,** T cells and viral persistence: lessons from diverse infections. *Nat. Immunol.* 2005. **6**: 873–879.

7 **Belkaid, Y. and Rouse, B. T.,** Natural regulatory T cells in infectious disease. *Nat. Immunol.* 2005. **6**: 353–360.

8 **Coffer, P. J. and Burgering, B. M.,** Forkhead-box transcription factors and their role in the immune system. *Nat. Rev. Immunol.* 2004. **579**: 191–198.

9 **Fontenot, J. D., Rasmussen, J. P., Williams, L. M., Dooley, J. L., Farr, A. G. and Rudensky, A. Y.,** Regulatory T cell lineage specification by the forkhead transcription factor Foxp3. *Immunity* 2005. **22**: 329–341.

10 **Sakaguchi, S.,** Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat. Immunol.* 2005. **6**: 345–352.

11 **Wu, Y., Borde, M., Heissmeyer, V., Feuerer, M., Lapan, A. D., Stroud, J. C., Bates, D. L. *et al.*,** FOXP3 controls regulatory T cell function through cooperation with NFAT. *Cell* 2006. **126**: 375–387.

12 **Seddiki, N., Santner-Nanan, B., Tangye, S. G., Alexander, S. I., Solomon, M., Lee, S., Nanan, R. and de Saint Groth, B. F.,** Persistence of naive CD45RA^{+} regulatory T cells in adult life. *Blood* 2006. **107**: 2830–2838.

13 **Thornton, C. A., Upham, J. W., Wikstrom, M. E., Holt, B. J., White, G. P., Sharp, M. J., Sly, P. D. and Holt, P. G.,** Functional maturation of CD4^{+}CD25^{+} CTLA4^{+}CD45RA^{+} T regulatory cells in human neonatal T cell responses to environmental antigens/allergens. *J. Immunol.* 2004. **173**: 3084–3092.

14 **Bacchetta, R., Passerini, L., Gambineri, E., Dai, M., Allan, S. E., Perroni, L., Dagna-Bricarelli, F. *et al.*,** Defective regulatory and effector T cell functions in patients with FOXP3 mutations. *J. Clin. Invest.* 2006. **116**: 1713–1722.

15 **Tsunemi, S., Iwasaki, T., Imado, T., Higasa, S., Kakishita, E., Shirasaka, T. and Sano, H.,** Relationship of CD4^{+}CD25^{+} regulatory T cells to immune status in HIV- infected patients. *AIDS* 2005. **19**: 879–886.

16 **Aandahl, E. M., Michaelsson, J., Moretto, W. J., Hecht, F. M. and Nixon, D. F.,** Human CD4^{+} CD25^{+} regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. *J. Virol.* 2004. **78**: 2454–2459.

17 **Eggena, M. P., Barugahare, B., Jones, N., Okello, M., Mutalya, S., Kityo, C., Mugyenyi, P. and Cao, H.,** Depletion of regulatory T cells in HIV infection is associated with immune activation. *J. Immunol.* 2005. **174**: 4407–4414.

18 **Weiss, L., Donkova-Petrini, V., Caccavelli, L., Balbo, M., Carbonneil, C. and Levy, Y.,** Human immunodeficiency virus-driven expansion of CD4^{+} CD25^{+} regulatory T cells, which suppress HIV-specific CD4 T-cell responses in HIV-infected patients. *Blood* 2004. **104**: 3249–3256.

19 **Kinter, A. L., Hennessey, M., Bell, A., Kern, S., Lin, Y., Daucher, M., Planta, M. *et al.*,** CD25(+)CD4(+) regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4(+) and CD8(+) HIV-specific T cell immune responses *in vitro* and are associated with favorable clinical markers of disease status. *J. Exp. Med.* 2004. **200**: 331–343.

20 **Andersson, J., Boasso, A., Nilsson, J., Zhang, R., Shire, N. J., Lindback, S., Shearer, G. M. and Chougnet, C. A.,** The prevalence of regulatory T cells in lymphoid tissue is correlated with viral load in HIV-infected patients. *J. Immunol.* 2005. **174**: 3143–3147.

21 **Krathwohl, M. D., Schacker, T. W. and Anderson, J. L.,** Abnormal presence of semimature dendritic cells that induce regulatory T cells in HIV-infected subjects. *J. Infect. Dis.* 2006. **193**: 494–504.

22 **Ndhlovu, L. C., Loo, C. P., Spotts, G., Nixon, D. F. and Hecht, F. M.,** FOXP3 expressing CD127lo CD4^{+}T cells inversely correlate with CD38^{+}CD8^{+} T cell activation level in primary HIV-1 infection. *J. Leukoc. Biol.* 2008. **83**: 254–262.

23 **Chase, A. J., Yang, H., Zhang, H., Blankson, J. N. and Siliciano, R. F.,** Preservation of FOXP3^{+} regulatory T cells in the peripheral blood of HIV-1-infected elite suppressors correlates with low CD4^{+} T-cell activation. *J. Virol.* 2008. **82**: 8307–8315.

24 **de St Groth, B. F. and Landay, A. L.,** Regulatory T cells in HIV infection: pathogenic or protective participants in the immune response? *AIDS* 2008. **22**: 671–683.

25 **Keynan, Y., Card, C. M., Mclaren, P. J., Dawood, M. R., Kasper, K. and Fowke, K. R.,** The role of regulatory T cells in chronic and acute infections. *Clin. Infect. Dis.* 2008. **46**: 1046–1052.

26 **Sallusto, F., Lenig, D., Forster, R., Lipp, M. and Lanzavecchia, A.,** Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999. **401**: 708–712.

27 **Lim, H. W., Broxmeyer, H. E. and Kim, C. H.,** Regulation of trafficking receptor expression in human forkhead box P3+regulatory T cells. *J. Immunol.* 2006. **177**: 840–851.

28 **Wei, S., Kryczek, I. and Zou, W.,** Regulatory T-cell compartmentalization and trafficking. *Blood* 2006. **108**: 426–431.

29 **Seddiki, N., Santner-Nanan, B., Martinson, J., Zaunders, J., Sasson, S., Landay, A., Solomon, M. *et al.*,** Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J. Exp. Med.* 2006. **203**: 1693–1700.

30 **Liu, W., Putnam, A. L., Xu-Yu, Z., Szot, G. L., Lee, M. R., Zhu, S., Gottlieb, P. A. *et al.*,** CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4^{+}Treg cells. *J. Exp. Med.* 2006. **203**: 1701–1711.

31 **Sousa, A. E., Carneiro, J., Meier-Schellersheim, M., Grossman, Z. and Victorino, R. M.,** CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J. Immunol.* 2002. **169**: 3400–3406.

32 **Epple, H. J., Loddenkemper, C., Kunkel, D., Troger, H., Maul, J., Moos, V., Berg, E. *et al.*,** Mucosal but not peripheral FOXP3^{+} regulatory T cells are highly increased in untreated HIV infection and normalize after suppressive HAART. *Blood* 2006. **108**: 3072–3078.

33 **Hazenberg, M. D., Stuard, J. W., Otto, S. A., Borleffs, J. C., Boucher, C. A., de Boer, R. J., Miedema, F. and Hamann, D.,** T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). *Blood* 2000. **95**: 249–255.

34 **Vukmanovic-Stejic, M., Zhang, Y., Cook, J. E., Fletcher, J. M., McQuaid, A., Masters, J. E., Rustin, M. H. *et al.*,** Human CD4^{+} CD25^{hi} Foxp3^{+} regulatory T cells are derived by rapid turnover of memory populations *in vivo*. *J. Clin. Invest.* 2006. **116**: 2423–2433.

Abbreviations: **ART:** antiretroviral therapy · **HIV-1:** HIV type 1 · **IRS:** immune reconstitution syndrome · **Tcon:** conventional CD4^{+} T cells

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針刺し(曝露)事故時のHIV感染症に関する対応についてのアンケート調査結果と対策

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要　旨

北陸3県（富山，石川，福井）の病院での針刺し(曝露)事故時のHIV感染症に対する対応について，299病院の薬剤師を対象にアンケート調査を行なった．その結果，127病院で針刺し事故が起きており，針刺し事故時のHIV感染症に関しても不安を持っていることが明らかになった．アンケートの結果を踏まえ，当院の針刺し（曝露）事故後の対応や北陸3県の行政機関の感染予防対策について，アンケート実施病院にアンケート結果と共に情報提供した．針刺し（曝露）事故後の対策は，予防薬剤の配置の有無で3県間に違いはあるが，それぞれの実情にあった体制作りを継続する必要がある．

（石川県中医誌30：9－12，2008）

キーワード：HIV，針刺し（曝露）事故，アンケート調査

緒　言

石川県立中央病院は1997年よりエイズ治療北陸ブロック拠点病院として，最新の医療の提供と共に，地域の病院に対して講演会や連絡会等で情報提供を行い，連携に努めている．HIV感染者やAIDS患者は年々増加しており，厚生労働省エイズ動向委員会は2007年の新規のHIV感染者は1,048名，AIDS患

表1：「針刺し(曝露)事故時のHIV感染症に関する対応」アンケート

所在地	所在地[ ]富山県	[ ]石川県	[ ]福井県
病床数	[ ]床		
貴院が掲げている診療科目すべてに○印をつけてください．	[ ]内科	[ ]小児科	[ ]眼科
	[ ]産婦人科	[ ]歯科	[ ]歯科口腔外科
	[ ]外科	[ ]形成外科	[ ]整形外科
	[ ]心臓血管外科	[ ]呼吸器外科	[ ]脳神経外科
	[ ]泌尿器科	[ ]皮膚科	[ ]放射線科
	[ ]麻酔科	[ ]耳鼻咽喉科	[ ]リハビリテーション科
	[ ]肛門科	[ ]その他（ ）	

＊＊　以下の設問で当てはまるもののカッコ内に○をつけて下さい．　＊＊

貴院の状況を教えて下さい．

1．針刺し(曝露)事故が発生した場合，現在行っているHIV感染予防についての対応を教えて下さい．（複数回答可）
[ ] 院内で対応，処理している
[ ] 問題があれば拠点病院に相談している
[ ] 問題があれば保健所へ相談している
[ ] 決まっていない
[ ] 対応していない
[ ] その他（ ）

2．針刺し(曝露)事故時のHIV対応マニュアルはありますか．
[ ] ある
[ ] ない
[ ] 作成中
[ ] その他（ ）

3．針刺し(曝露)事故時に備えて予防薬剤※を準備していますか．（※予防薬剤：針刺し(曝露)事故後に早めに内服することでHIV感染を防止するといわれている薬剤）
[ ] 準備している
[ ] 準備していない
[ ] 検討中
[ ] その他（ ）

4－1．予防薬剤を「準備している」病院にお聞きします．
針刺し(曝露)事故予防薬剤の薬剤名を教えて下さい．予防のために準備している薬剤すべてに○をつけて下さい．
[ ] レトロビル(AZT) [ ] エピビル(3TC) [ ] ビラセプト(NFV)
[ ] クリキシバン(IDV) [ ] カレトラ (LPV/RTV)
[ ] その他（ ）

4－2．予防薬剤を「準備していない」病院にお聞きします．
準備していない理由を教えて下さい．（複数回答可）
[ ] HIV/エイズ患者がいないので不必要
[ ] 抗HIV薬は高価なので不良在庫となるため
[ ] 設置の必要性を感じない（理由： ）
[ ] その他（ ）

5．平成18年1月－12月の間に針刺し(曝露)事故が発生しましたか．
[ ] あった
[ ] なかった
[ ] わからない

6．平成18年に「針刺し(曝露)事故があった」病院にお聞きします．
その件数を教えて下さい．また，そのうちHIV感染を疑ったのは何件ですか．
[ ]件　そのうちHIV感染を疑った件数は[ ]件

7．針刺し(曝露)事故の後にHIV予防薬を服用したケースはありましたか．
[ ] ある
[ ] ない
[ ] 不明

8．針刺し(曝露)事故後の対応について困っていること，不安に思っていること，提案等がありましたら自由にお書き下さい．また，予防薬剤の準備(備蓄)についてお考えがあればお聞かせ下さい．

協力ありがとうございました．

締切日：平成19年1月22日（月）

*1　薬剤部
*2　財団法人エイズ予防財団
*3　診療部（血液免疫内科）

者は400名と報告している．一方，医療機関ではHIV抗体検査が日常的には行われていない現状がある．HIV汚染血液による針刺しでのHIV感染率は約0.3%[1]で，粘膜曝露では約0.09%[2]と報告されている．また，HIVは曝露後なるべく早く抗HIV薬を内服することで感染のリスクを減らすことができると言われている[3]．しかし，抗HIV薬には副作用が強いものや胎児に対して安全性が確立されていない薬もあり，注意が必要である．また，抗HIV薬は高価であり，石川県下では使用頻度が少ないこともあり，緊急の購入は困難な薬剤でもある．そこで，北陸3県の針刺し(曝露)事故時の対応などの現状を把握し，問題点を明らかにすることで対応・体制整備の一助とするためアンケートを行なったので報告する．

対象および方法

アンケートは2007年1月に北陸3県の299病院の薬剤師を対象に郵送法で行なった(表1)．

結　果

回答率は63.9%で，富山県は58.0%，石川県は65.4%，福井県は67.5%で3県に差はなかった（表2）．

針刺し事故が発生した場合，現在行っているHIV感染予防についての対応では，院内で対応，処理しているが69病院，問題があれば拠点病院に相談しているが77病院，問題があれば保健所へ相談しているが27病院，決まっていないは45病院，対応していないは20病院であった（図1）．

針刺し事故時のHIV感染予防対応マニュアルは約半数の100病院にあるが，針刺し事故時に備えて予防薬剤を準備している病院は24病院であった．予防薬剤の最も多い組み合わせはＡＺＴ＋３ＴＣ＋ＮＦＶで，17病院がこの薬剤を準備していた（図２，３）．予防薬剤を「準備していない」病院の準備していな

表2：アンケートの回答状況

	調査病院数	回答病院数	回答率(%)
富山県	112	65	58.0
石川県	107	70	65.4
福井県	80	54	67.5
記入なし		2	
合　計	299	191	63.9

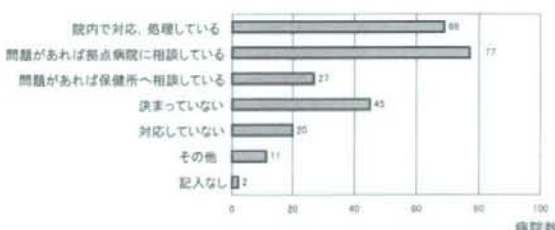

図1：針刺し(曝露)事故が発生した場合のHIV感染予防に関する対応（複数回答可）

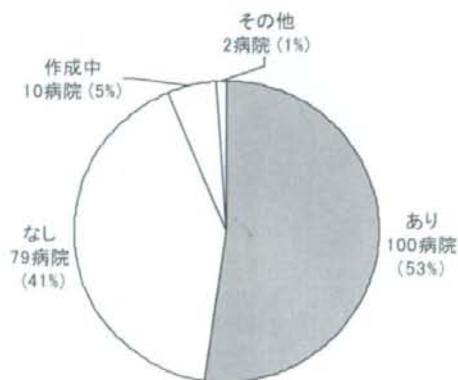

図2：針刺し(曝露)事故時のHIV対応マニュアルの有無

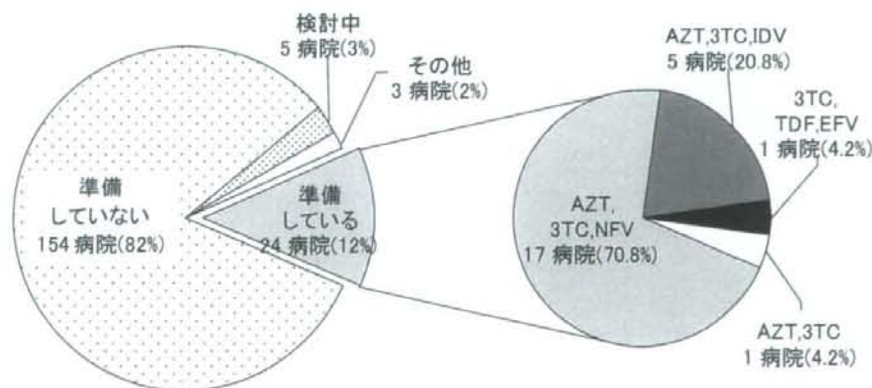

図3：針刺し(曝露)事故に備えて予防薬剤を準備しているか，またその薬剤は何か

い理由は，患者がいないので不必要が80病院，不良在庫になるためが50病院であった（図4）．その他，拠点病院や専門医を受診するので不必要，検査を実施していない，マニュアルがない，高齢者の病院なので必要ない，検討中という回答があった．

2006年1月～12月の間に針刺し事故が発生した病院は127病院であった．この127病院での件数の内訳は，3件以内が半数を占めていたが，21件以上が8病院あった．最も多かった件数は43件であった．この127病院での針刺し事故でHIV感染を疑ったのは7病院の30件であった（図5，6，表3）．

針刺し事故が起こった127病院のうち実際に抗HIV薬の予防内服を行なった施設は7病院であった（図7）．

アンケートで針刺し事故後の対応について困っていることや，不安に思っていること，予防薬剤の備蓄についての考えなどを自由記載として尋ねたところ，58病院から回答があり，キーワードで検査，備蓄薬剤，マニュアル，不安，その他に分けた（表4）．最も多かったのは検査について（16病院）であり，患者のHIV検査を行なっていないのでどう対処すればよいかであった．その他，薬剤の備蓄にはコストがかかる，B型肝炎やC型肝炎のマニュアルはあるがHIVはない，また針刺し事故があっても報告されていない事も多いのではないか等の意見があった．

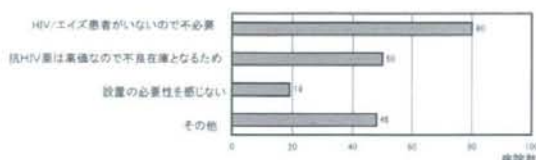

図4：予防薬剤を「準備していない」病院の準備していない理由（複数回答可）(165施設の回答)

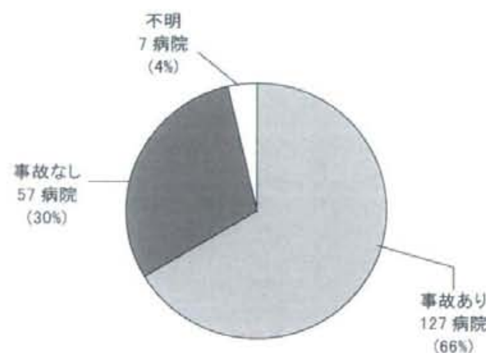

図5：2006年1月～12月の間に針刺し(曝露)事故の発生の有無

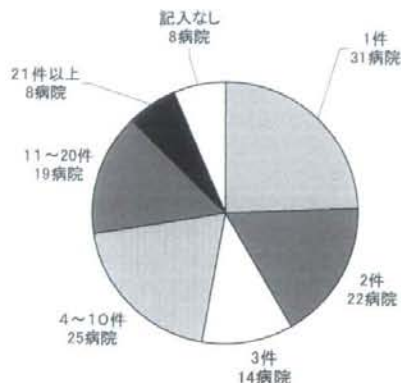

図6：2006年に「針刺し(曝露)事故があった」病院の事故件数

表3：2006年に「針刺し(曝露)事故があった」病院でHIV感染を疑った件数

HIV疑い件数	
0件	108病院
1件	5病院
10件	1病院
15件	1病院
記入なし	12病院

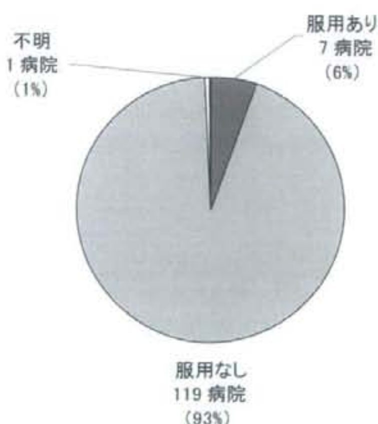

図7：2006年に「針刺し(曝露)事故があった」病院でHIV予防薬を服用しケースはあるか

表4：アンケートでの自由記載（抜粋）

58病院の意見

検査について（16病院）

- HIV結果不明の方の針刺し事故はすべてHIV（＋）としての対応が必要なのか．
- 患者のHBV，HCVについては，容易に検査可能であるが，HIVについては患者のプライバシーの問題もあり事実不可能である．他施設の対応を知りたい．
- 針刺事故は保険で対処できないため様々な検査ができていない（患者も職員もエイズであるかどうか）

備蓄薬剤について（15病院）

- HIV感染症の治療をおこなっていない病院では予防薬剤を常時準備しておくことはかなり難しいと思う
- 薬剤の備蓄にはコストが大変かかる．保健所などでの備蓄を希望する．など

マニュアルについて（15病院）

- B型，C型肝炎のマニュアルは存在する．近い将来HIVのマニュアルも作成していきたい．
- HIVに関する資料が手元にない．他病院のマニュアルを借りたい．など

不安など（7病院）

- HIVは不安を抱きながらも対応できてない．
- エイズ拠点病院がどのような協力をどれだけしてもらえるか等，拠点病院との連携のありかたが不明確である．など

その他　（5病院）

- 高齢者のみの病院だが，10年以内にHIV感染者も入ってくる可能性がある．もう3～5年したら60代の患者さんの入院時HIVの検査が必要と思ってる．
- 事故の発生があっても報告されていない事が多いのではないか．報告しづらい為なのか…報告の必要性を周知させる必要がある．など

考　察

今回のアンケート調査では，127病院で針刺し事故が起こっており，アンケートの回答を得た191病院の66%であった．事故後のHIV感染を疑った病院は7病院と少なかったが，58病院に自由記載があり，針刺し事故後のHIV感染予防に関心が高いことが伺われた．「HIV感染を疑っていない」という回答の中には患者のHIV検査をしていないので感染したかどうかがわからない，予防薬剤がないので疑っても服薬できないなどの不安も含まれていると推察できる．また，79病院（41%）に針刺し事故時のHIV対応マニュアルがなく，「HIVに関する資料がない」，「他の病院のマニュアルを借りたい」などの意見もあり情報提供の必要が伺われた．予防薬剤を備蓄している病院は24病院（12%）と少なかった．備蓄しない理由はHIV/AIDS患者がいないので不必要，抗HIV薬は高価なので不良在庫になるためが多かったが，拠点病院を受診するので不必要という回答もあった．また，針刺し事故が発生した場合，「拠点病院に相談する」という回答は77病院（40%）と多く，拠点病院は周辺の病院に対し，針刺し事故時の相談や予防措置等でどのような協力ができるかを示すことが望まれる．

2007年2月に開催された「北陸HIV臨床談話会」でアンケート結果を報告し，拠点病院の医療従事者間で針刺し事故時の対応を検討した．また，アンケート結果を299の病院に通知する際に，北陸3県の行政機関の針刺し事故時の対応や当院の針刺し事故時の対応(フローチャート)についての情報提供を行なった．

北陸3県の行政機関の針刺し（曝露）事故時の対応は，2007年1月時点での各県の担当者に確認したものであるが，以下のとおりである．富山県では2つの拠点病院に受診し，予防薬の処方を受ける．福井県では近くの拠点病院に相談する．石川県では6病院に予防薬を設置している．当院でも石川県の行政機関から予防薬の配置があり，貸出も可能である．

以上のように，北陸3県の針刺し（曝露）事故後の対策については，各県で違いはあるが，それぞれの実情にあった体制作りを継続する必要があると思われる．

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文　献

1）Bell DM：Occupational risk of human inmmunodeficiency virus infection in healthcare workers, an overview. Am J Med 102：9−15, 1997

2）Ippolito G et al：The risk of occupational human innunodeficiency virus infection in health care workers. Arch Intern Med 153：1451−1458, 1993

3）小林寛伊ほか：医療従事者における刺傷事故について－日本の多施設実態調査より－．環境感染11：1−6, 1996

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Human Herpesvirus 8 DNA Load in the Leukocytes Correlates with the Platelet Counts in HIV Type 1-Infected Individuals

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Abstract

Human herpes virus 8 (HHV-8) is known to be reactivated in immunocompromised situations and it is associated with Kaposi's sarcoma (KS) and some hematological diseases. The aim of this study was to analyze the effect of HHV-8 on HIV-1 infection, especially on thrombocytopenia complicated with HIV infection. The HHV-8 DNA load was determined by a quantitative real-time PCR, using leukocytes from 125 HIV-1-infected individuals. HHV-8 DNA was detected in 37 individuals. The increased HIV-1 load and reduced percentage of CD4-positive T cells were significantly associated with the presence of HHV-8. The prevalence and load for HHV-8 are higher in patients with KS than in patients without KS, but the difference is not significant. The increased HHV-8 DNA load was significantly correlated with thrombocytopenia, and platelet counts were significantly lower in individuals with HHV-8 than in individuals without HHV-8. We also obtained the negative correlations between changes in platelet counts and changes in HHV-8 DNA loads. The association between thrombocytopenia and HHV-8 has never been reported previously, apart from some case reports of Castleman's disease and KS. Various cytokines or chemokines are produced by HHV-8-infected cells, some of which have been reported to inhibit hematopoiesis. This may be one of the mechanisms by which HHV-8 infection induces thrombocytopenia. These results indicate that HHV-8 DNA in leukocytes may provide useful information for the assessment of the clinical appearance of HIV-1 infection.

Introduction

HHV-8, A NEW MEMBER OF THE GAMMAHERPESVIRINAE, was identified as the etiologic agent of Kaposi's sarcoma (KS). The main transmission routes of human herpes virus 8 (HHV-8) seem to be sexual contact,[1] but transmission by saliva,[2] blood products, and organ graft[3,4] has also been proposed. The distribution of HHV-8 is related to a combination of geographic and behavioral risk factors. Serological studies have shown that HHV-8 seroprevalence is high in Africa and the Middle East and low in Europe and the United States. In Japan, it is reported that HHV-8 seroprevalence among healthy controls is 0.2–1.4% and HHV-8 seroprevalence among HIV-1-positive homosexual men is 11.6–63.6%.[5,6]

HHV-8 can infect circulating B cells, monocytes, macrophages, T cells, and KS-like spindle cell progenitors,[7–9] and usually persists in a latent state in these cells. The reactivation of this latent HHV-8 infection can be induced by a number of conditions, including superinfection by other viruses, stress, chronic illnesses, malignancies, and immunosuppressive disorders, such as HIV infection. HHV-8 contains more than 80 open reading frames, including several homologues of oncogenes, cytokine, and cytokine response genes. During latent and lytic infection, some viral genes are expressed and play a causative role in the genesis of some diseases, such as AIDS and non-AIDS-related KS, multicentric Castleman's disease, body cavity-based lymphoma, and some lymphoproliferative diseases. Our previous study reported a case of Castleman's disease with HIV-1 infection in which repeated episodes of thrombocytopenia were correlated with an increase in HHV-8 DNA loads in leukocytes.[10] The aim of this study was to determine the prevalence and loads of HHV-8 DNA in peripheral blood leukocytes in HIV-1-positive individuals and to investigate the correlation with the clinical appearance of HIV-1 infection, especially with thrombocytopenia.

Materials and Methods

Patients and samples

All consecutive HIV-1-infected patients who attended Kyushu Medical center between April 2005 and August 2006

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were eligible for this study. The exclusion criteria were HCV infection, active hepatitis, and treatment for antiherpes therapy at the time of sampling. Nine HIV-1-positive individuals were analyzed longitudinally before and after the initiation of antiretroviral therapy (ART). For controls, 12 HIV-1-seronegative patients with autoimmune thrombocytopenia and 17 HIV-1-seronegative healthy volunteers were sampled for baseline comparisons. Informed consent for blood sampling was obtained from all participants. The study was conducted according to the ethical guidelines of the hospital and was approved by an authorized representative of the hospital. EDTA-treated blood was taken from the subjects, and leukocytes were collected after removing red blood cells with hemolysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA). Cell pellets were stored at −20°C until use.

Real-time quantitative PCR

DNA was extracted from the cell pellets using a QIAamp Blood Mini kit (QIAGEN Inc., Tokyo, Japan). Real-time PCR was conducted with the LineGene33 (BioFlux, Tokyo, Japan) using Premix Ex Taq (TAKARA, Shiga, Japan). As an internal control measurement, to normalize for input DNA, copy numbers of ß$_2$-microglobulin (ß$_2$M) were determined in every sample tested. The primers used for amplification were as follows: HHV-8 forward, 5′-CCTCTGGTCCCCATTCATTG-3′, and reverse, 5′-CGTTTCCGTCGTGGATGAG-3′, and probe 5′-FAM-CCGGCGTCAGACATTCTCACAACC-TAMRA-3′;[11] Epstein–Barr virus (EBV) forward, 5′-CGGAAGCCCTCTGGA CTTC-3′, and reverse, 5′-CCCTGTTTATCCGATGGAATG – 3′, and probe 5′-FAM-TGTACACGCACGAGAAATGCGCC-TAMRA-3′;[12] ß $_2$M forward, 5′-CAGCAAGGACTGGTCTTT CTATCTCT-3′, and reverse, 5′-ACCCCACTTAACTATCTT GG-3′, and probe 5′-FAM-CACTGAAAAAGATGAGTATG CCTGCCGTGT-TAMRA-3′.[13] Standards were obtained by amplification of a control sample in a polymerase chain reaction (PCR) reaction using the same primers. The data were normalized as copies/10^6 cells by measuring copy numbers of the ß$_2$M gene, since two ß$_2$M copy numbers correspond to one cell. The lower limit of detection was defined as 1 copy/10^6 cells.

Statistical analyses

Frequency analysis was performed using Fisher's exact test for 2×2 tables. The viral DNA copy numbers of HHV-8, EBV, and HIV-1 were $\log_{10}$ transformed and compared between different groups by means of the Mann–Whitney *U* test. The associations between them were determined using an analysis of covariance (ANCOVA), with EBV-DNA and HIV-RNA as covariates, where HHV-8 DNA was adjusted on the bases of EBV-DNA and HIV-RNA. Spearman's rank correlation coefficient was used to compare changes in platelet counts and changes in HHV-8 DNA loads. A partial correlation was used to assess this relationship, while controlling for any changes in HIV-RNA and EBV-DNA.

Results

Subjects characteristics

A total of 125 patients fulfilled the inclusion criteria. Demographic and clinical characteristics of the subjects included in this study are shown in Table 1. There were 8 women and 117 men, 112 of whom were men who have sex with men; the others were heterosexual. These patients were ranged from 20 to 69 years of age (mean age, 37.8 years). Of these, 58 received ART and 67 remained untreated. The duration of ART was 3 months to 9.5 years (mean 3.25 years). Nine HIV-1-positive individuals were analyzed longitudinally before and after the initiation of ART. Among 125 HIV-1-positive subjects included in our study, we evaluated 97 subjects for the prevalence of splenomegaly by means of an abdominal ultrasound examination; 23 subjects were thus found to have splenomegaly.

HHV-8 qualitative and quantitative DNA analysis

The presence and load of HHV-8 DNA were investigated in 125 HIV-1-positive subjects and 17 HIV-1-negative healthy controls (Fig. 1). HHV-8 DNA was detected in 37 of 125 (29.6%) leukocyte samples from HIV-1-positive subjects, with values ranging from 2 to 91,171 copies/10^6 leukocytes. HHV-8 DNA was not detected in any healthy controls.

Relationship between HHV-8 DNA and KS

HHV-8 DNA was detected in three of five (60%) HIV-infected subjects with KS, whereas they were found in 34 of 120 (28.3%) subjects without KS ($p = 0.15$). Figure 2 shows that the HHV-8 DNA load of subjects with KS to be higher than that of the subjects without KS, but not significantly so.

The correlation between HHV-8 DNA and HIV-1-related immunovirological parameters

The HIV-RNA load in the serum of HHV-8 DNA-positive subjects was significantly higher than that of HHV-8 DNA-negative subjects (Fig. 3a). In addition, the correlation between HHV-8 DNA and hypergammaglobulinemia (the percentage of gammaglobulin is more than normal range) was examined, which is often observed in HIV-1-positive subjects. The HHV-8 DNA load in leukocytes in subjects with

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF 125 HIV-POSITIVE SUBJECTS[a]

	Total	*Pretreatment of ART*	*Treatment of ART*	P
N (male/female)	125 (117/8)	67 (63/4)	58 (54/4)	
Age (years)	37.8 ± 10.4	34.3 ± 8.2	42.1 ± 11.2	<0.001
CD4 (cells/ml)	396 ± 218	387 ± 226	407 ± 211	0.616
Viral load (copies/ml) ($\log_{10}$)		4.36 ± 0.86	Under detection limit	
Duration of ART (years)		0	3.25 ± 2.68	

[a]The mean values ± SE are shown. Pretreatment of ART versus treatment of ART.