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.

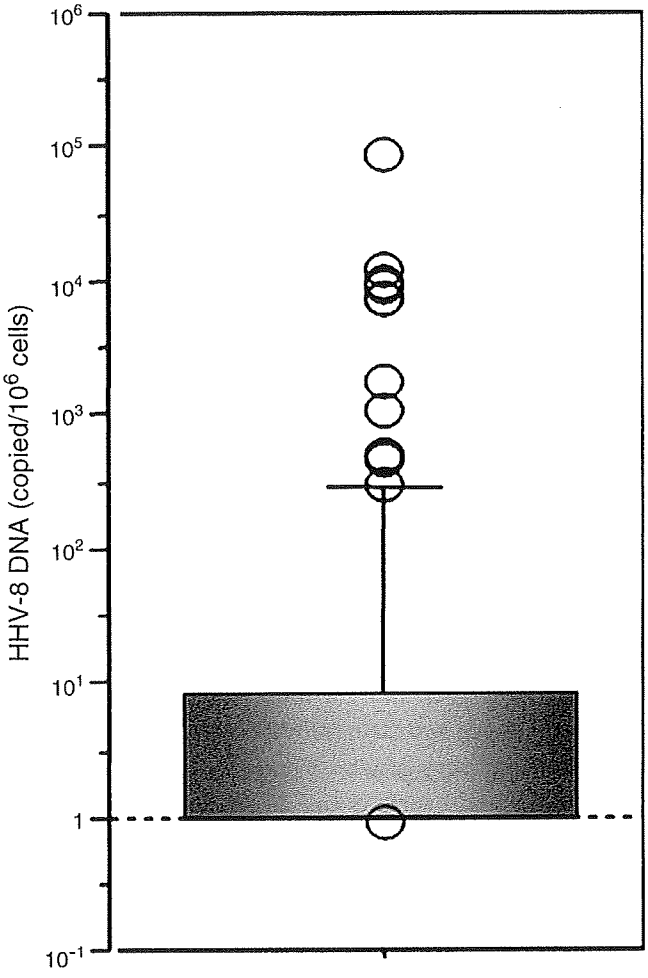

FIG. 1. Quantification of HHV-8 DNA by real-time PCR. Log_{10}-transformed HHV-8 copy numbers per 10^6 leukocytes are shown. The bar shows the mean and standard deviations. The dotted line shows the detection limit of the assay.

hypergammaglobulinemia was significantly higher than in subjects without hypergammaglobulinemia (Fig. 3b). HHV-8 DNA was detected in 24 of 44 (54.5%) HIV-infected subjects with hypergammaglobulinemia, whereas it was found in 13 of 81 (16.0%) subjects without hypergammaglobulinemia ($p < 0.0001$). We could not find any significant correlation between HHV-8 DNA loads and CD4-positive T cell counts.

The effect of ART on HHV-8 DNA

HHV-8 DNA load in leukocytes in subjects with ART was significantly lower than in subjects without ART (Fig. 4a). HHV-8 DNA was detected in 12 of 58 (20.7%) HIV-infected subjects with ART, whereas it was found in 25 of 67 (37.3%) subjects without ART ($p = 0.042$). There was no difference in the duration of ART between HHV-8 DNA-negative and HHV-8 DNA-positive subjects with ART (3.161 ± 2.764 vs. 3.575 ± 2.439 years, $p = 0.624$). HHV-8 DNA loads (log_{10}) per 10^6 leukocytes in subjects with a short duration of ART (<6 months) were higher than in subjects with a long duration of ART (≥6 months), but the difference was not statistically significant (0.758 ± 0.447 vs. 0.323 ± 0.111, $p = 0.309$).

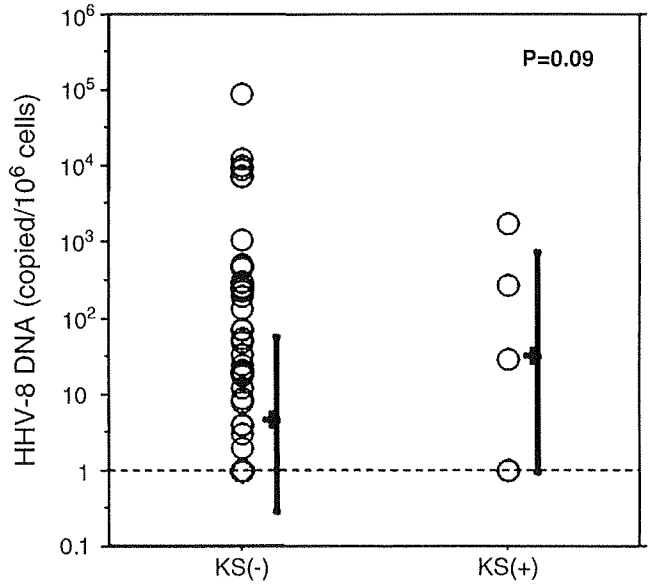

FIG. 2. HHV-8 DNA loads among the HIV-1-positive subjects with or without KS. Log_{10}-transformed HHV-8 copy numbers per 10^6 leukocytes are shown. The bar shows the mean and standard deviations. p-values were estimated by the Mann–Whitney U test. The dotted line shows the detection limit of the assay.

Longitudinal analysis of HHV-8 DNA loads after initiation of ART

The longitudinal profiles of HHV-8 DNA loads were examined in nine subjects after initiation of ART (Fig. 4b). Within 1 month after starting ART, HHV-8 DNA transiently increased in three patients (Fig. 4b, black straight lines); in two of the three patients, KS had progressed transiently. These findings show the reactivation of HHV-8 by initiation of ART. Three months after the initiation of ART, all nine patients had achieved and maintained the decreased levels of HHV-8 DNA.

The influence of EBV on HHV-8 infection

Like HHV-8, EBV is lymphotropic and is a member of the gammaherpesvirinae; it is reactivated by HIV-1 infection. Therefore, the influence of EBV on HHV-8 infection was investigated. EBV-DNA loads (log_{10}) per 10^6 leukocytes were significantly higher in HHV-8 DNA-positive subjects (0.69 ± 0.11 vs. 1.46 ± 0.21, $p = 0.0007$) and EBV DNA was detected in 32 of 88 (36.4%) HHV-8-negative subjects, whereas they were found in 25 of 37 (67.5%) HHV-8-positive subjects ($p = 0.002$).

Relationship between HHV-8 DNA and thrombocytopenia

Figure 5 shows a case of Castleman's disease in which the platelet counts changed in inverse association with HHV-8 DNA in the leukocytes. Therefore, the association between platelet counts and HHV-8 DNA in leukocytes was examined. First, the changes in platelet counts and HHV-8 DNA were analyzed in the nine subjects before and after the initiation of ART (Fig. 6). Both the decrease in HHV-8 DNA and the

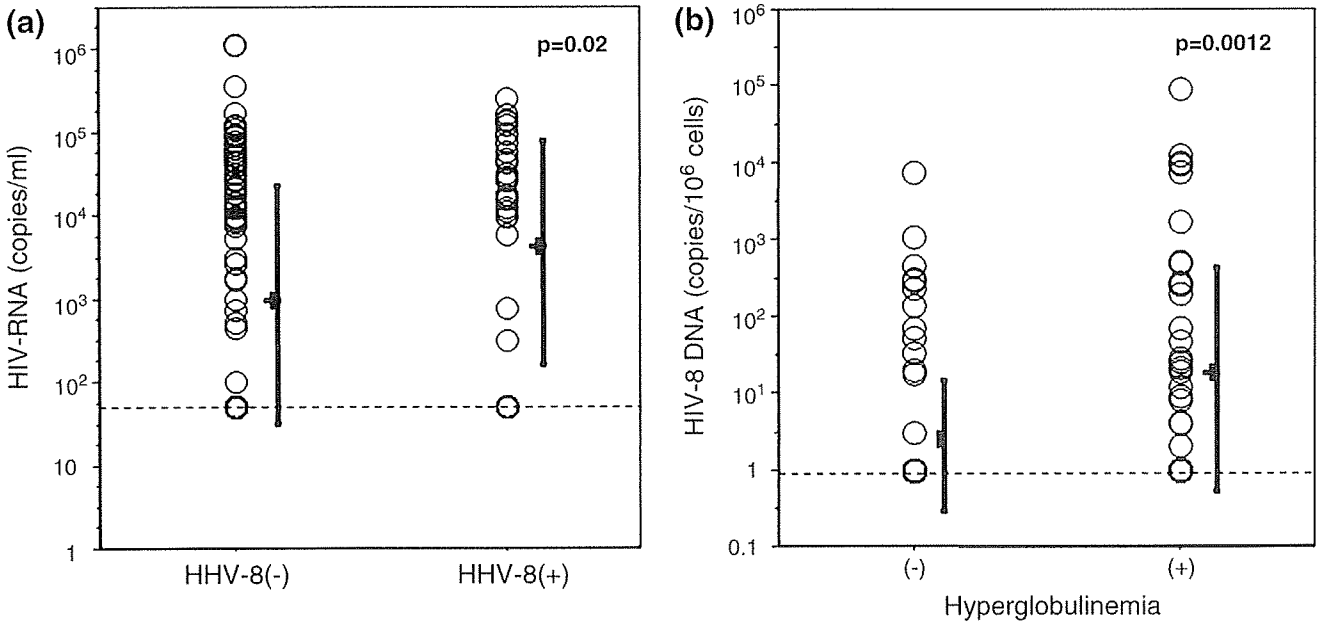

FIG. 3. The correlation between HHV-8 DNA and HIV-1-related immunovirological parameters. (a) HIV-RNA loads among the HIV-1-positive subjects with or without HHV-8 DNA. Log_{10}-transformed HIV-1 copy numbers/ml of serum are shown. The bar shows the mean and standard deviation. p-values were estimated by the Mann–Whitney U test. (b) HHV-8 DNA loads among the HIV-1-positive subjects with or without hypergammaglobulinemia. HHV-8 copy numbers per 10^6 leukocytes are shown. The bar shows the mean and standard deviations. p-values were estimated by the Mann–Whitney U test. The dotted line shows the detection limit of the assay.

increase in platelet counts were significant. Furthermore, the correlation between changes in platelet counts and changes in HHV-8 DNA was obtained (Spearman; $r = -0.783$, $p = 0.0267$). Patients with HIV infection sometimes develop thrombocytopenia, and EBV is also reported to cause mild thrombocytopenia. Therefore, partial correlations were also used to assess this relationship while controlling for EBV-DNA and HIV-RNA, and the correlation between the change in HHV-8 DNA and platelet counts was obtained (partial correlation coefficient; $r = -0.733$, $p = 0.0219$).

Next, all 125 subjects with HIV infection were analyzed. There was no significant difference between the group with

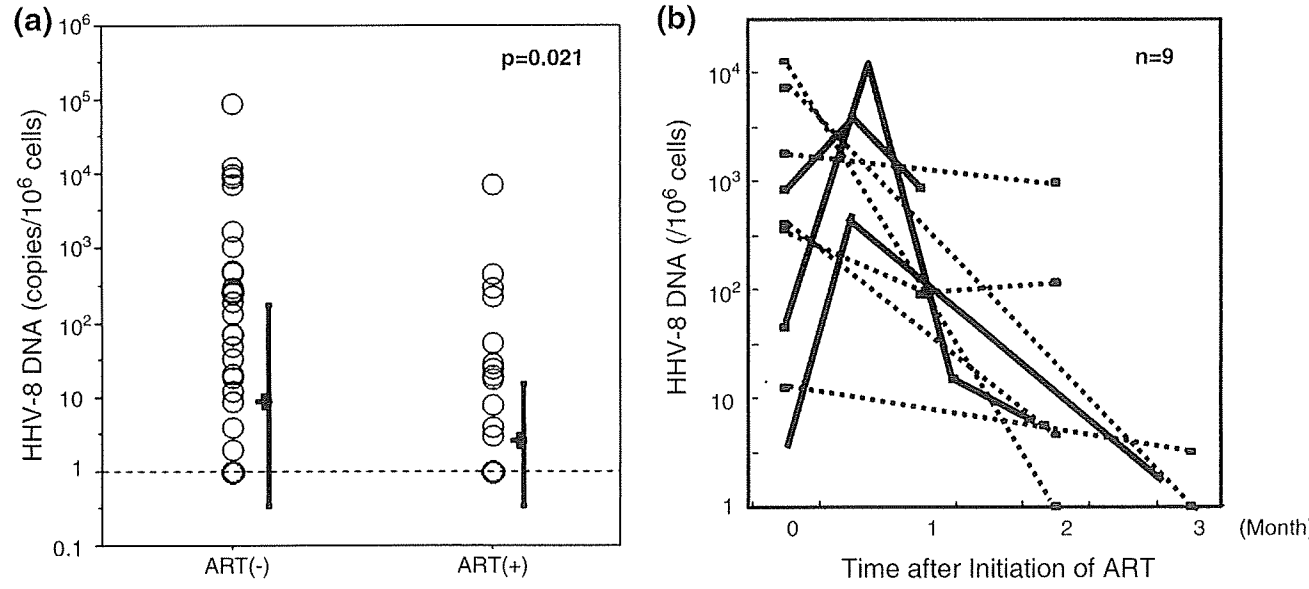

FIG. 4. The effect of ART on HHV-8 DNA. (a) HHV-8 DNA load in PBMCs in subjects with or without ART. HHV-8 copy numbers per 10^6 leukocytes are shown. The bar shows the mean and standard deviation. p-values were estimated by the Mann–Whitney U test. (b) HHV-8 DNA load after initiation of ART. HHV-8 DNA loads of the nine subjects were measured at the indicated time points. The dotted line shows the detection limit of the assay.

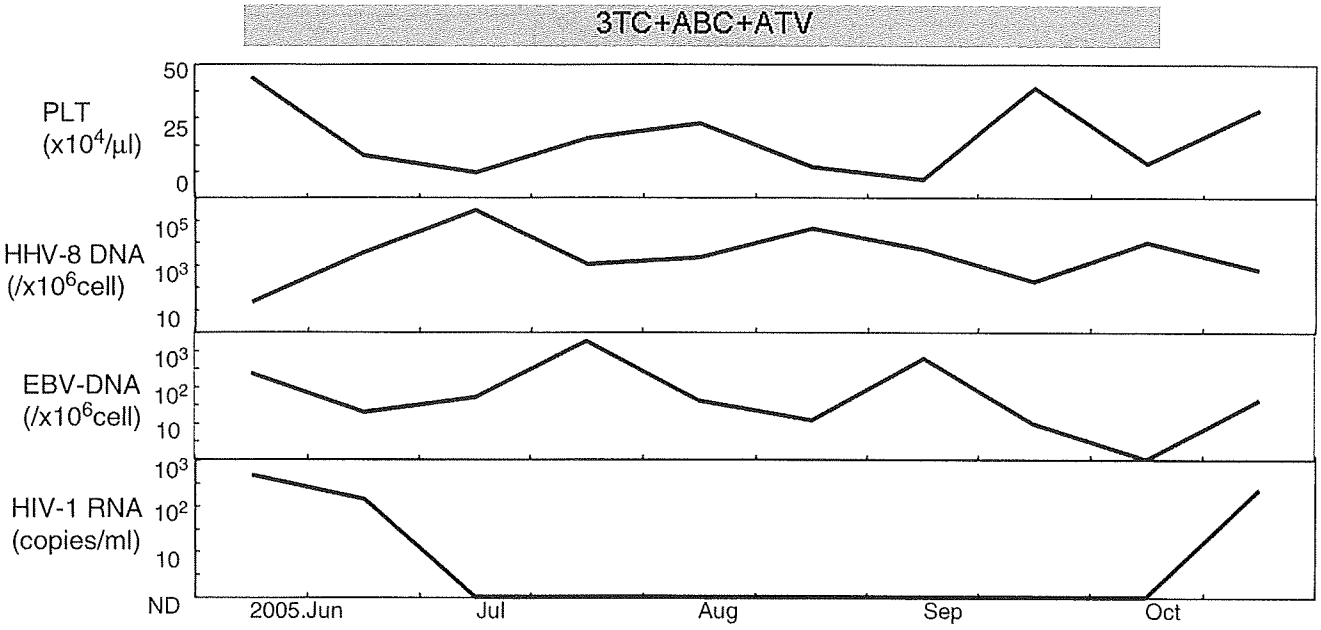

FIG. 5. Time course of the case of Castleman's disease with HIV-1 infection. Platelet counts, $\log_{10}$-transformed HHV-8 and EBV copy numbers per 10^6 leukocytes, and $\log_{10}$-transformed HIV-1 copy numbers/ml of serum are shown. The data were excerpted from Minami and Yamamoto.[10] PLT, platelet; HHV-8, human herpes virus 8; EBV, Epstein–Barr virus; 3TC, lamivudine; ABC, abacavir; ATV, atazanavir.

thrombocytopenia and the group without thrombocytopenia in terms of the number of subjects with ART, the duration of ART, and the prevalence of subjects with splenomegaly (with thrombocytopenia vs. without thrombocytopenia; 30.8% vs. 22.6%, $p = 0.520$). Similarly, no significant difference was observed between the group with HHV-8-DNA and the group without HHV-8-DNA in terms of the duration of ART and the prevalence of subjects with splenomegaly (with HHV-8 vs. without HHV-8; 26.7% vs. 22.4%, $p = 0.647$). The effects of HHV-8 DNA in leukocytes on platelet counts were examined using ANCOVA, with EBV-DNA as a covariate. ANCOVA revealed a significant interaction between EBV-DNA and HHV-8 DNA but no significant interaction between thrombocytopenia and EBV-DNA. Post hoc tests revealed that HHV-8 DNA was significantly higher in subjects with platelet counts <150,000 platelets/μl than in subjects with platelet counts ≥150,000 platelets/μl (Fig. 7a). HHV-8 DNA was detected in 11 of 14 (78.6%) subjects with low platelet counts,

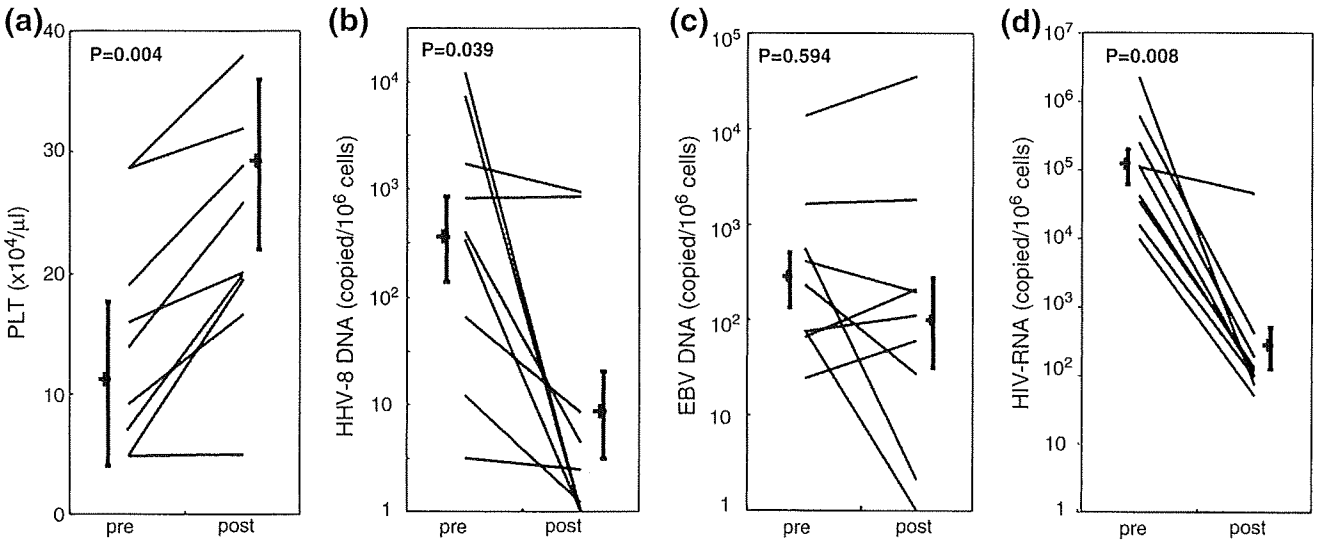

FIG. 6. Changes in the platelet counts, HHV-8 DNA loads, EBV-DNA loads, and HIV-RNA of pre- and post-ART. Platelet counts (a), HHV-8-DNA (b), EBV-DNA (c), and HIV-RNA (d) of the nine subjects were analyzed before (pre) and after 2 or 3 months of initiation of ART (post). The viral loads were $\log_{10}$ transformed. Bar shows the mean and standard deviation. p-values for the difference between the two time points were estimated by the Wilcoxon signed rank test.

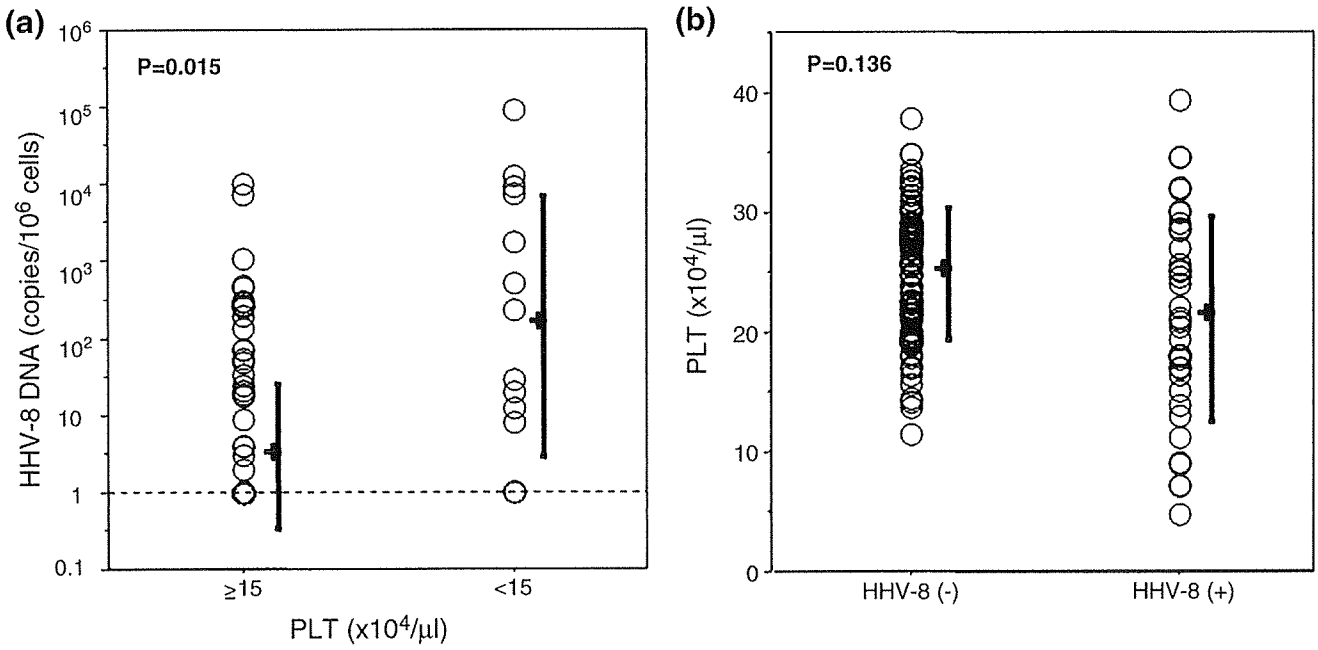

FIG. 7. Relationship between HHV-8 DNA and mild thrombocytopenia.(**a**) HHV-8 DNA load in leukocytes in subjects with platelet counts of <150,000 platelets/mm^3 or with platelet counts of ≥150,000 platelets/mm^3. HHV-8 copy numbers per 10^6 leukocytes are shown. The bar shows the mean and standard deviation. The *p*-values were estimated by a post hoc test after ANCOVA with EBV-DNA as a covariate. (**b**) Platelet counts among the HIV-1-positive subjects with or without HHV-8 DNA. The bar shows the mean and standard deviation. The *p*-values were estimated by a post hoc test after ANCOVA with EBV-DNA as a covariate. The dotted line shows the detection limit of the assay.

whereas it was found in 26 of 111 (23.4%) of subjects with normal platelet counts ($p = 0.0059$: estimated by post hoc test after ANCOVA with the prevalence of EBV-DNA as a covariate). The platelet counts were lower in HHV-8 DNA-positive subjects than in HHV-8 DNA-negative subjects, *but not significanlyt* (Fig. 7b). In subjects without ART, HIV-RNA might be a confounding factor, and thus ANCOVA was conducted with HIV-RNA and EBV-DNA as covariates.

In subjects without ART, HHV-8 DNA was also significantly higher in subjects with mild thrombocytopenia with an adjustment for EBV-DNA and HIV-RNA ($p = 0.013$, data not shown). HHV-8 DNA was not detected in the 12 subjects with autoimmune thrombocytopenia. We also examined the correlation between HHV-8 DNA loads and platelet counts in subjects without KS, because the subject with KS might have occult Castleman's disease, which can cause thrombocytopenia. HHV-8 DNA was significantly higher in subjects with platelet counts of <150,000 platelets/μl than in subjects with platelet counts of ≥150,000 platelets/μl [$\log_{10}$ (HHV-8) = 2.103 ± 1.797 vs. 0.438 ± 0.924; $p = 0.0285$]. HHV-8 DNA was detected in 9 of 12 (75.0%) subjects with low platelet counts, whereas it was found in 25 of 108 (23.1%) subjects with normal platelet counts ($p = 0.0002$). The platelet counts were significantly lower in HHV-8 DNA-positive subjects than in HHV-8 DNA-negative subjects (21.5 ± 8.61/μl vs. 25.0 ± 5.56/μl; $p = 0.1319$).

Discussion

Some previous studies have examined the prevalence of HHV-8 infection in HIV-1-infected subjects. A unique aspect of this study is that the effect of HHV-8 DNA in the peripheral blood leukocytes on HIV-1 infection was examined; a significant association between HHV-8 DNA and the clinical appearance of HIV-1 infection, especially thrombocytopenia complicated with HIV-1, was found.

Some reports have shown that the presence of KS was associated with the prevalence of HHV-8 DNA in leukocytes[14] and the severity was associated with HHV-8 DNA loads in leukocytes.[15] The current data also showed a certain association with the presence of KS and HHV-8 DNA load, but in this study there were only five subjects with KS, because the prevalence of KS in HIV-1-infected patients in Japan is low. As a result, it was impossible to determine whether the HHV-8 DNA load predicted the onset and severity of KS. To draw any conclusions concerning the clinical and prognostic utility on KS, more subjects with KS need to be analyzed.

HHV-8 encodes homologues of cytokine and cytokine response genes, such as viral interleukin-6 (vIL-6)[16] and viral interferon regulatory factor (vIRF). vIL-6 can bind the gp130 receptor to activate IL-6 response genes and promote B cell activation, which is the mechanism of hypergammaglobulinemia induced by HHV-8 infection. It was also found that HHV-8 plays an important role in hypergammaglobulinemia, which is often found in subjects with HIV-1 infection.

The current data showed that HIV-RNA load in the serum of HHV-8 DNA-positive subjects was significantly higher than that found in the serum of HHV-8 DNA-negative subjects. HHV-8 replication is activated by HIV-Tat,[17] and it has been reported that HHV-8 reactivation is associated with HIV-1 acute infection.[18] Furthermore, an open reading frame (ORF), the major transactivator of the HHV-8 lytic cycle, can also induce increased levels of HIV replication. In conclusion, these findings show that HHV-8 coinfection is associated with

immunological characteristics and disease severity of HIV infection.

ART reduced HHV-8 DNA loads as well as HIV-RNA (Fig. 4). The inhibition of HIV-1 replication with ART probably leads to the reconstitution of the immune system and regenerates effective immune responses against HHV-8. Moreover, protease inhibitors block the production of inflammatory cytokines,[19] which in turn may result in the downregulation of HHV-8 replication. In this longitudinal study during ART, HHV-8 DNA loads transiently increased in three subjects soon after the initiation of ART. This might be due to the disorder of cytokine balance, which occurs transiently in the process of the reconstitution of the immune system, and this might be one of the explanations for the immune reconstitution syndrome of KS. Some inflammatory cytokines are induced in immune reconstitution syndromes. It has recently been reported that some cytokines, which are associated with the development of KS, were elevated in other reconstitution syndromes.[20] It is possible that reactivation of HHV-8 by ART might activate the inflammation of the other reconstitution syndromes by the increase in vIL-6 or other cytokines.

The difference between HHV-8 DNA in the subjects with platelet counts of <150,000 platelets/μl and in the subjects with platelet counts of ≥150,000 platelets/μl was significant, but the difference between the platelet counts in the HHV-8 DNA-positive subjects and the platelet counts in the HHV-8 DNA-negative subjects was not significant. This is because HHV-8 DNA correlates with "mild" thrombocytopenia.

This is the first report that analyzed the association between HHV-8 DNA and thrombocytopenia in HIV-1-infected subjects, though there are a few studies that showed the relationship between HHV-8 infection and hematological disorders[21] or posttransplantation bone marrow failure in HIV-1-negative subjects.[22] Some case reports have described thrombocytopenia complicated with HHV-8-induced Castleman's disease, hemophagocytic syndrome, or other inflammatory syndromes. The HIV-1-positive subjects sometimes develop thrombocytopenia, which has been thought to be caused by an autoimmune mechanism. Recently, antibody cross-reactivity between a known epitope region of HIV-1 proteins and platelet GPIIIa49-66 has been reported to be responsible for HIV-1-associated thrombocytopenia.[23] However, the present study showed that HHV-8 also contributes independently to the thrombocytopenia complicated in HIV-1 infection.

The molecular mechanism of thrombocytopenia induced by HHV-8 remains to be established, but it is possible that some cytokines or chemokines induced by HHV-8 might be involved in thrombocytopenia. For example, interleukin-8 (IL-8), which is induced by HHV-8 infection,[24] binds the receptors on megakaryocytes and platelets and inhibits megakaryocytopoiesis.[25] Furthermore, HHV-8 is reported to infect CD34-positive hematopoietic progenitor cells[26,27]; HHV-8 harboring CD34-positive cells might also contribute to hematological disorders including thrombocytopenia.[28] Another possible explanation for thrombocytopenia is an autoimmune mechanism such as immune thrombocytopenia (ITP). Chronic stimulation of the B cell clones induced by HHV-8 could favor the production of autoantibodies, some of which might react against platelet. In fact, Evan's syndrome complicating multicentric Castleman's disease[29] and ITP complicating KS have been reported.[30]

In Italy, the area of subendemicity of HHV-8, the presence of HHV-8 DNA in HIV-1-negative elderly people has been reported to be associated with mild thrombocytopenia.[31] In Japan, HHV-8 infection in HIV-1-negative subjects is rare; as a result, when the 12 subjects with autoimmune thrombocytopenia were examined, the association between HHV-8 and autoimmune thrombocytopenia in HIV-1-negative subjects was not distinct. However, the fact that HHV-8 DNA was not detected in the HIV-1-negative subjects with autoimmune thrombocytopenia suggests that HHV-8 is not a main causative agent of autoimmune thrombocytopenia.

In conclusion, a significant association was observed between HHV-8 infection and the clinical appearance of HIV-1 infection, especially in regard to mild thrombocytopenia, hypergammaglobulinemia, and disease severity. It is possible that some of these effects of HHV-8 infection are related to other independent etiological cofactor or some other viruses. However, it may be useful to monitor HHV-8 DNA in leukocytes of HIV-1-positive subjects to assess and predict disease severity and to select the optimal treatment modalities.

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Disclosure Statement

No competing financial interests exist.

References

1. Ablashi DV, Chatlynne LG, Whitman JE Jr, and Cesarman E: Spectrum of Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8, diseases. Clin Microbiol Rev 2002; 15:439–464.
2. Miller CS, Berger JR, Mootoor Y, Avdiushko SA, Zhu H, and Kryscio RJ: High prevalence of multiple human herpesviruses in saliva from human immunodeficiency virus-infected persons in the era of highly active antiretroviral therapy. J Clin Microbiol 2006;44:2409–2415.
3. Challine D, Roudot-Thoraval F, Sarah T, *et al.*: Seroprevalence of human herpes virus 8 antibody in populations at high or low risk of transfusion, graft, or sexual transmission of viruses. Transfusion 2001;41:1120–1125.
4. Stein L, Carrara H, Norman R, Alagiozoglou L, Morris L, and Sitas F: Antibodies against human herpesvirus 8 in South African renal transplant recipients and blood donors. Transpl Infect Dis 2004;6:69–73.
5. Fujii T, Taguchi H, Katano H, *et al.*: Seroprevalence of human herpesvirus 8 in human immunodeficiency virus 1-positive and human immunodeficiency virus 1-negative populations in Japan. J Med Virol 1999;57:159–162.
6. Katano H, Iwasaki T, Baba N, *et al.*: Identification of antigenic proteins encoded by human herpesvirus 8 and seroprevalence in the general population and among patients with and without Kaposi's sarcoma. J Virol 2000;74:3478–3485.

7. Mesri EA, Cesarman E, Arvanitakis L, *et al.*: Human herpesvirus-8/Kaposi's sarcoma-associated herpesvirus is a new transmissible virus that infects B cells. J Exp Med 1996;183:2385–2390.
8. Sirianni MC, Vincenzi L, Topino S, *et al.*: Human herpesvirus 8 DNA sequences in CD8 + T cells. J Infect Dis 1997;176:541.
9. Sirianni MC, Uccini S, Angeloni A, Faggioni A, Cottoni F, and Ensoli B: Circulating spindle cells: Correlation with human herpesvirus-8 (HHV-8) infection and Kaposi's sarcoma. Lancet 1997;349:255.
10. Minami R and Yamamoto M: A case of HIV-1 and HHV-8-associated Castleman disease with a relapsing high fever and lymphoadenopathy. Kansenshogaku Zasshi 2006;80: 423–427.
11. Polstra AM, Van Den Burg R, Goudsmit J, and Cornelissen M: Human herpesvirus 8 load in matched serum and plasma samples of patients with AIDS-associated Kaposi's sarcoma. J Clin Microbiol 2003;41:5488–5491.
12. Kimura H, Morita M, Yabuta Y, *et al.*: Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. J Clin Microbiol 1999;37:132–136.
13. Nagai H, Wada K, Morishita T, Utsumi M, Nishiyama Y, and Kaneda T: New estimation method for highly sensitive quantitation of human immunodeficiency virus type 1 DNA and its application. J Virol Methods 2005;124:157–166.
14. Boivin G, Gaudreau A, Toma E, *et al.*: Human herpesvirus 8 DNA load in leukocytes of human immunodeficiency virus-infected subjects: Correlation with the presence of Kaposi's sarcoma and response to anticytomegalovirus therapy. Antimicrob Agents Chemother 1999;43:377–380.
15. Laney AS, Cannon MJ, Jaffe HW, *et al.*: Human herpesvirus 8 presence and viral load are associated with the progression of AIDS-associated Kaposi's sarcoma. AIDS 2007;21:1541–1545.
16. Aoki Y, Jones KD, and Tosato G: Kaposi's sarcoma-associated herpesvirus-encoded interleukin-6. J Hematother Stem Cell Res 2000;9:137–145.
17. Harrington W Jr, Sieczkowski L, Sosa C, *et al.*: Activation of HHV-8 by HIV-1 tat. Lancet 1997;349:774–775.
18. Lennette ET, Busch MP, Hecht FM, and Levy JA: Potential herpesvirus interaction during HIV type 1 primary infection. AIDS Res Hum Retroviruses 2005;21:869–875.
19. Wasmuth JC, Nischalke HD, Jütte A, *et al.*: Chemokine mRNA levels in mononucleated cells of HIV-infected patients before and after initiation of PI- versus NNRTI-containing HAART. Antiviral Res 2004;61:207–212.
20. Tamburini J, Grimaldi D, Chiche JD, Bricaire F, and Bossi P: Cytokine pattern in Kaposi's sarcoma associated with immune restoration disease in HIV and tuberculosis co-infected patients. AIDS 2007;21:1980–1983.
21. Tsai WH, Lee YM, Ing-Tiau Kuo B, *et al.*: Increased seroprevalence of human herpesvirus 8 in patients with hematological disorders. Acta Haematol 2005;114:95–98.
22. Cuzzola M, Irrera G, Iacopino O, *et al.*: Bone marrow failure associated with herpesvirus 8 infection in a patient undergoing autologous peripheral blood stem cell transplantation. Clin Infect Dis 2003;37:102–106.
23. Li Z, Nardi MA, and Karpatkin S: Role of molecular mimicry to HIV-1 peptides in HIV-1-related immunologic thrombocytopenia. Blood 2005;106:572–576.
24. Sun Q, Matta H, Lu G, and Chaudhary PM: Induction of IL-8 expression by human herpesvirus 8 encoded vFLIP K13 via NF-kappaB activation. Oncogene 2006;25:2717–2726.
25. Kowalska MA, Ratajczak J, Hoxie J, Brass LF, Gewirtz A, Poncz M, and Ratajczak MZ: Megakaryocyte precursors, megakaryocytes and platelets express the HIV co-receptor CXCR4 on their surface: Determination of response to stromal-derived factor-1 by megakaryocytes and platelets. Br J Haematol 1999;104:220–229.
26. Henry M, Uthman A, Geusau A, *et al.*: Infection of circulating CD34 + cells by HHV-8 in patients with Kaposi's sarcoma. J Invest Dermatol 1999;113:613–616.
27. Wu W, Vieira J, Fiore N, *et al.*: KSHV/HHV-8 infection of human hematopoietic progenitor (CD34+) cells: Persistence of infection during hematopoiesis *in vitro* and *in vivo*. Blood 2006;108:141–151.
28. Quinn JP, Gilligan OM, and Horgan M: Evan's syndrome complicating multicentric Castleman's disease—dramatic response to rituximab. Eur J Haematol 2004;73:384–385.
29. Brown EE, Whitby D, Vitale F, *et al.*: Correlates of human herpesvirus-8 DNA detection among adults in Italy without Kaposi sarcoma. Int J Epidemiol 2005;34:1110–1117.
30. Muela Molinero A, Ballesteros del Rio B, Sandoval Guerra V, and Llor Banos J: Evans syndrome as presentation of multicenter Castleman disease. Rev Clin Esp 2003;203:616–617.
31. Toyohama T, Nagasaki A, Miyagi J, *et al.*: Kaposi's sarcoma in a human immunodeficiency virus-negative patient treated with corticosteroid for idiopathic thrombocytopenic purpura. Intern Med 2003;42:448–449.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

High Molecular Weight Form of Adiponectin in Antiretroviral Drug-induced Dyslipidemia in HIV-Infected Japanese Individuals Based on *in vivo* and *in vitro* Analyses

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Abstract

Objective High molecular weight (HMW)-adiponectin has been found to be a better negative regulator of insulin resistance than total adiponectin. The aim of this study was to investigate the influence of HMW-adiponectin on antiretroviral therapy (ART)-induced dyslipidemia in Japanese human immunodeficiency virus (HIV)-infected individuals. We also examined the effect of some antiretroviral drugs (ARVs) on adipocytes *in vitro*.

Patients and Methods Fifty-seven HIV-infected patients were enrolled in four clinical groups; (I) patients who started ART containing efavirenz (EFV); (II) patients who started ART containing a protease inhibitor without atazanavir (ATV); (III) patients who started ART containing ATV; (IV) patients who switched from ART without ATV into ART containing ATV. We measured the serum HMW-adiponectin before and one year after starting or changing ART, using an enzyme-linked immunoSorbent assay (ELISA). Furthermore, we treated the mouse adipocytes (3T3-L1) with some ARVs. The lipid content was assessed using Oil Red O staining. The expression of adiponectin was measured by quantitative real-time PCR.

Results The serum HMW-adiponectin decreased significantly in groups (I) and (II) after starting ART, and increased significantly in group (IV) after changing from ART without ATV to ART with ART. EFV, ritonavir (RTV) and nelfinavir (NFV) inhibited the expression of adiponectin mRNA in mature 3T3-L1 and to a greater extent in pre-mature 3T3-L1. This phenomenon was reversible when ARV was changed to ATV.

Conclusion Effects of the ARVs on adiponectin may vary depending on the administration of different drugs. These data suggest that the distinct metabolic effects of ARV could therefore be a consequence of their differential effects on the production of adiponectin.

Key words: HMV-adiponectin, antiretroviral therapy, dyslipidemia

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Introduction

Adiponectin is an adipokine secreted exclusively by adipocytes: this protein plays an important role in the control of systematic lipid metabolism and insulin sensitivity (1). Adiponectin forms multimers and is present in the serum as a trimer, hexamer, or as a high molecular weight (HMW) form (2). The HMW isoform most avidly binds to its receptors and stimulates AMP-activated protein kinase, one of the key molecules mediating the metabolic actions of adiponectin.

Antiretroviral therapy (ART) has prolonged survival in human immunodeficiency virus (HIV)-infected individuals, but most individuals receiving ART develop metabolic abnormalities, which include dyslipidemia (elevated plasma triglycerides and cholesterol), increased visceral and dorsocervical adipose tissue and peripheral lipoatrophy (3). Although

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HIV infection itself (4) and nucleoside reverse transcriptase inhibitors (NRTI) (5-7) have been associated with metabolic abnormalities, there are increasing clinical and epidemiological data that suggest a central role for HIV protease inhibitors (PIs) in the causation of metabolic complications (8) and atazanavir (ATV) use has been associated with a decrease in hyperlipidemia, less insulin resistance and reversal of lipodystrophy (9).

Several recent clinical studies suggest that hypoadiponectinemia might play an important role in the causation of metabolic abnormalities associated with HIV infected individuals treated with PI-based ART, especially for those with lipodystrophy (8, 10, 11), but to date, limited data have been published whether this hypoadiponectimia is due to a disturbance of adipocyte differentiation, or to the decrease in the number of adipose cells number, or a dysfunction of adipocytes. In addition, there are still no data regarding the influence of antiretroviral drugs (ARVs) on serum HMW-adiponectin levels in Japanese, about 40% of whom have a genetic variation in the adiponectin gene associated with a reduced adiponectin level (12).

This study compared the effect on HMW-adiponectin level between ATV, PIs (except for ATV), ATV replacement therapy and non-nucleoside reverse transcriptase inhibitors (NNRTI), efavirenz (EFV) in HIV-infected Japanese subjects. In addition, the influence of each of the ARVs on adipocyte development and the expression of adiponectin were evaluated, using in vitro models.

Materials and Methods

Study population

The subjects evaluated in this study consisted of 57 HIV-positive patients admitted to National Hospital Organization Kyushu Medical Center, who started ART or were changing the ART combination. The present study was conducted in accordance with the regulations of the institutional ethics committee. These individuals were enrolled into four clinical groups; (I) patients who started ART containing EFV; (II) patients who started ART containing PIs without ATV; (III) patients who started ART containing ATV; (IV) patients who replaced the ART without ATV into ART containing ATV. The Body Mass Index (BMI), serum triglyceride, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, HMW adiponectin were measured before and one year after starting or changing ART. HMW-adiponectin was measured by enzyme-linked immunosorbent Assay (ELISA), using the Human Adiponectin ELISA kit for Total and Multimers (Daiichi Pure Chemicals Co., Tokyo, Japan). Lipodystrophy was determined by a standardized, lipodystrophy-specific physical examination which recorded lipoatrophy and/or diffuse fat accumulation in the face, neck, dorsocervical spine, arms, breasts, abdomen, buttocks and legs.

Cells

The 3T3-L1 cells were purchased from the Japanese Collection of Research Bioresources. The cells were maintained in DMEM supplemented with 10% fetal bovine serum. For differentiation, post confluent cells were induced by incubation with 0.5 mM 3-isobutyl-methylxanthine and 1 μM dexamethasone for 2 days. This is followed by incubation with 10 μg/mL insulin for 2days. The cells were then maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) for another 2 days. To investigate the effects of ARVs on adipogenesis, and to investigate the difference between the effect of ARVs on preadipocytes and the effect on mature adipocytes, several ARVs were added to the medium before and after differentiation of 3T3-L1 cells.

Antiretroviral drugs

Efavirenz (EFV), ritonavir (RTV), nelfinavir (NFV) were purchased from Toronto Research Chemicals Inc. (Ontario, Canada). ATV was provided by Bristol-Myers Squibb Company (NY, USA). Drug stocks in dimethyl sulfoxide (DMSO) were stored at -20°C and diluted into culture media. Vehicle control incubations received the same final DMSO concentration as all drug-treated incubations (0.1%). The IC50 /IC95 and the Cmax of each drug are as follows; EFV (IC95=1.7-25nM, Cmax= 14.2-28.8 μM), RTV (IC50= 65-289nM, Cmax= 0.84-21.9 μM), NFV (IC50=30-60nM, Cmax= 5.0-8.6 μM), and ATV (IC50=2-5nM, Cmax=4.96-8.38 μM). The cells were treated with 20 μM of EFV, RTV and ATV and 10 μM of NFV.

Quantitative real time RT-PCR

Total cellular RNA was isolated from 3T3-L1 cells, using QIAamp RNA Blood Mini (QIAGEN, Tokyo, Japan), including treatment with DNase. cDNA was generated from the RNA using TAKARA RNA PCR kit (TAKARA BIO, Shiga, Japan). Real time PCR was conducted with the LineGene33 (BioFlux, Tokyo, Japan) using SYBR Green Realtime PCR Master Mix (TOYOBO Co, Osaka, Japan). As an internal control measurement, to normalize for input DNA, copy numbers of β-actin were determined in every sample tested. The ratio of the normalized mean value for drug-treated samples was calculated and is given in the graphs.

Measurement of adiponectin levels in culture medium

The secretion of adiponectin from 3T3-L1 cells were determined by measuring the adiponectin concentration of culture medium, using the Mouse Adiponectin/Acrp30 Immunoassay (R&D systems, Mimmeapolis, USA).

Oil Red O staining

The cellular lipid content was assessed by lipid staining with Oil Red O. Staining was quantified at 520 nm after

Table 1. Characteristics of 57 HIV-infected Individuals and the Changes of Metabolic Markers

	group I	group II	group III	group IV (pre)	group IV (post)
Number of cases (M/F)	15 (13/2)	14 (13/1)	14 (13/1)	14 (12/2)	
Mean age (SD) (years)	37.7 (10.8)	42.1 (14.2)	38.3 (9.1)	46.1 (12.6)	
NRTI combination (number)(frequency %)					
Abacavir/ lamivudine	0 (0)	0 (0)	1 (7.1)	0 (0)	2 (14.3)
Zidovudine/ lamivudine	4 (26.7)	0 (0)	4 (28.6)	5 (35.7)	4 (28.6)
Stavudine/ lamivudine	7(46.7)	7 (50)	5 (35.7)	6 (42.9)	4 (28.6)
Didanosine/ lamivudine	0 (0)	0 (0)	0 (0)	2 (14.3)	2 (14.3)
Didanosine/ zidovudine	0 (0)	2 (14.3)	0 (0)	0 (0)	0 (0)
Tenofovir/ emtricitabine	4 (26.7)	5 (35.7)	4(28.6)	1 (7.1)	2 (14.3)
the ratio of after to bebore staring or changing ART [mean (SE)]					
Body Mass Index	1.00 (0.01)	0.99 (0.01)	1.02 (0.01)	0.98 (0.04)	
Tryglyceride	1.63 (0.19)*	1.72 (0.24)*	1.22 (0.17)	1.00 (0.304)	
HDL-cholesterol	1.29 (0.12)*	1.17 (0.16)	1.14 (0.11)	1.01 (0.07)	
LDL-cholesterol	1.17 (0.12)	1.27 (0.12)*	1.10 (0.05)	0.85 (0.06)*	
HMW-adiponectin	0.61 (0.24)*	0.65 (0.09)*	1.19 (0.20)	1.61 (0.11)*	

P values are evaluated by one sample sign test. * indicates significant difference (* $p<0.001$, ** $p<0.05$)
NRTI, nucleotide reverse transcriptase inhibitor; ART, antiretroviral therapy
"pre" indicates before changing ART, and "post"indicates after changing ART.

solubilization, using an Adipogenesis Assay Kit (Chemicon International Temecula, CA, USA)

Statistical analysis

To evaluate the changes of BMI, serum triglyceride, LDL-cholesterol, HDL-cholesterol, HMW-adiponectin after starting or changing ART, the one sample sign test was applied. The Mann-Whitney test was used to compare the serum HMW-adiponectin according to lipodystrophy. In vitro experiments were reproduced in at least three independent experiments. The results are presented as the mean ± SD. Significance was determined as described in the figure legends.

Results

The effect of ARV on the lipid profile and HMW adiponectin

The demographic and clinical characteristics for the 57 patients included in the study are shown in Table 1. There were no significant differences in the age and the NRTI combinations used as the backbone of PIs or EFV among the four groups (χ-square test). The serum triglycerides and LDL-cholesterol increased significantly in individuals in groups I and II and LDL-cholesterol decreased significantly in individuals with group IV. The serum HMW-adiponectin decreased significantly in individuals in groups I and II and increased significantly in individuals with group IV. On the other hand, there was no change in the triglyceride, LDL-cholesterol, and HMW-adiponectin levels in individuals in group III (Table 1). These results show that serum HMW-adiponectin level decreased in individuals with ART including EFV or PIs except ATV, but the decrease was reversible and it was recovered by changing the ART into that including ATV.

HMW-adiponectin and lipodystrophy

The development of lipodystrophy was observed in 14 of the 43 patients who newly initiated ART (the patients of groups I, II, and III). Lipodystrophy was more prevalent in group I (53.3%) and group II (28.6%) than in group III (14.3%). The differences in lipodystrophy were not driven by the overall weight gain. There was no significant difference in the serum HMW-adiponectin before ART between individuals with and without lipodystrophy followed by ART, but the serum HMW-adiponectin after starting ART and the ratio of HMW-adiponectin after to before starting ART decreased significantly in individuals with lipodystrophy (Fig. 1).

The effect of ARV on adiponectin mRNA levels in differentiating 3T3-L1 cells

As shown in Table 1, the effect of ART on dyslipidemia and lipodystrophy differed among the ART menus, but it is nearly impossible to fully separate the effects of the drug classes in the clinical data, because the patients received a combination of several classes of ART. As a result, *in vitro* models were used to examine the precise influence of these drugs on adipocyte development or metabolism, using well-characterized preadipocyte 3T3-LI cells. The concentrations of ARVs used in this assay were within the range (RTV, EFV) or a little higher (ATV, NFV) than what is generally observed in plasma from individuals receiving therapeutic doses of ARV. Considering that some ARVs can accumulate in fat tissue, it is possible that the effects of ARVs on 3T3-L 1 cell lines observed *in vitro* may also occur *in vivo*. When pre-adipocytes were treated with ARV, lipid accumulation was severely reduced by EFV and NFV (Fig. 2A). The adiponectin mRNA level was reduced by RTV, EFV, NFV, and was not affected by ATV (Fig. 2B). Similar results were obtained concerning the secretion of adiponectin into the culture medium (Fig. 2C). Since it is possible that the effect of ARV on the adipocyte metabolism is known to differ between preadipocytes and mature adipocytes, the mature adipocytes were also treated with ARVs. Mature adipocytes were less sensitive to ARVs' effects on lipid accumulation and adiponectin mRNA level than premature adipocytes. Lipid accumulation was reduced by EFV, RTV and NFV (Fig. 2A). The adiponectin mRNA level was reduced by RTV, EFV, and NFV, whereas ATV did not affect the adi-

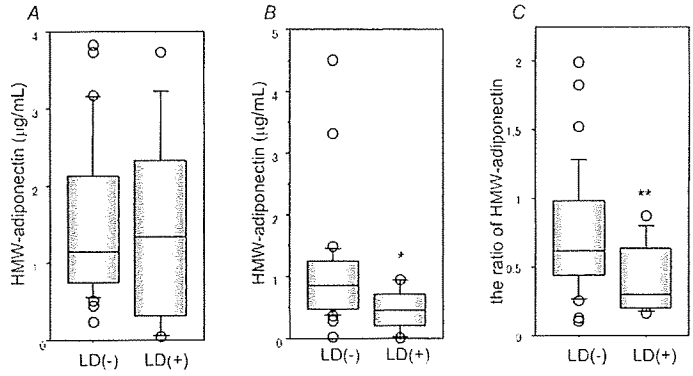

Figure 1. The levels of serum HMW-adiponectin in HIV-infected individuals with/without lipodystrophy. (A) HMW-adiponectin before starting ART, (B) HMW-adiponectin after starting ART, (C) the ratio of HMW-adiponectin after starting ART to that before starting ART. The medians are indicated with horizontal bars. The vertical bars indicate the range between 10% and 90% and the horizontal boundaries of the boxes represent the interquartile range. P values are evaluated by the Mann-Whitney U-test. * $p<0.01$, ** $p<0.05$ versus individuals without lipodystrophy. LD: lipodystrophy

ponectin mRNA level (Fig. 2B). Similar results were obtained concerning the secretion of adiponectin into the culture medium (Fig. 2C).

The effect of changing PI/EFV to ATV on adiponectin mRNA levels in 3T3-L1 cells

In the clinical study, the decreased adiponectin induced by PI/EFV was restored by switching the drugs to ATV. In addition, the precise effect of ATV on decreased adiponectin mRNA induced by PI/EFV was examined in vitro, using 3T3L1 cells. Adiponectin mRNA recovered significantly in mature adipocytes after switching drugs from EFV and NFV to ATV, but it did not recover on EFV- and NFV-treated pre-adipocytes and RTV-treated pre- and mature adipocytes (Fig. 3).

Discussion

Several studies have reported that metabolic syndrome is more common in subjects with HIV infection than in HIV-negative individuals. Although uncontrolled HIV replication can cause an adverse modification of the lipid metabolism, these modifications can be mainly induced by ART. The present study described the lipid abnormalities and lipodystrophy associated with ART. Hypertriglyceridemia was more common than abnormalities of HDL cholesterol and LDL cholesterol. LDL cholesterol increases were only observed in the population of group II (PI without ATV). These abnormalities were recovered by changing ART to the ATV containing regimen. In HIV-negative populations, the most common features associated with metabolic syndrome are obesity and hypertension. On the other hand, in HIV-positive populations, hypertriglyceridemia and hypertension are reported to be common components and the most frequent abnormalities that lead to metabolic syndrome (3). That is why HIV-positive patients might need to have their ART regimen tailored to their lipid abnormalities.

In this study, we also investigated the effect of ARVs on serum HMW-adiponectin, which has been reported to be a useful marker for evaluating insulin resistance and metabolic syndrome. This is the first study to investigate the influence of ARVs on the serum HMW-adiponectin levels in Japanese HIV-infected individuals. In general, the serum adiponectin levels are known to be inversely related to the adipose tissue mass (13). The serum adiponectin levels have been reported to rise when obese persons lose weight (14). We showed that adiponectin levels are relatively low in HIV-infected individuals with lipodystrophy who have a low fat mass, and this is consistent with previous reports (7, 10). These results suggest that the normal relationships between adiponectin concentration and adipose droplets appear to be lost or reversed in HIV-infected individuals. Since adiponectin expression is higher in subcutaneous fat than in visceral fat in humans (15), visceral fat accumulation and subcutaneous fat loss may thus lead to decreased adiponectin production both in lipoatrophic and lipohypertrophic patients. Therefore, fat redistribution may actually be responsible for the decreased adiponectin levels in HIV patients with lipodystrophy. As shown in Fig. 1(C), it is certain that some of the patients without lipodystrophy had a decrease in serum HMW-adiponectin levels, but these patients had dyslipidemia more frequently than the patients with either normal or high serum HMW-adiponectin without lipodystrophy (data not shown). Considering the fact that clinical HIV lipodystrophy has been reported to be associated with dyslipidemia, these patients might thus have the potential to be complicated by lipodystrophy. Therefore, the low levels of serum HMW-adiponectin are correlated with lipodystrophy and/or dyslipidemia induced by PIs and NNRTI (EFV).

The present study also showed the direct effects of ARVs

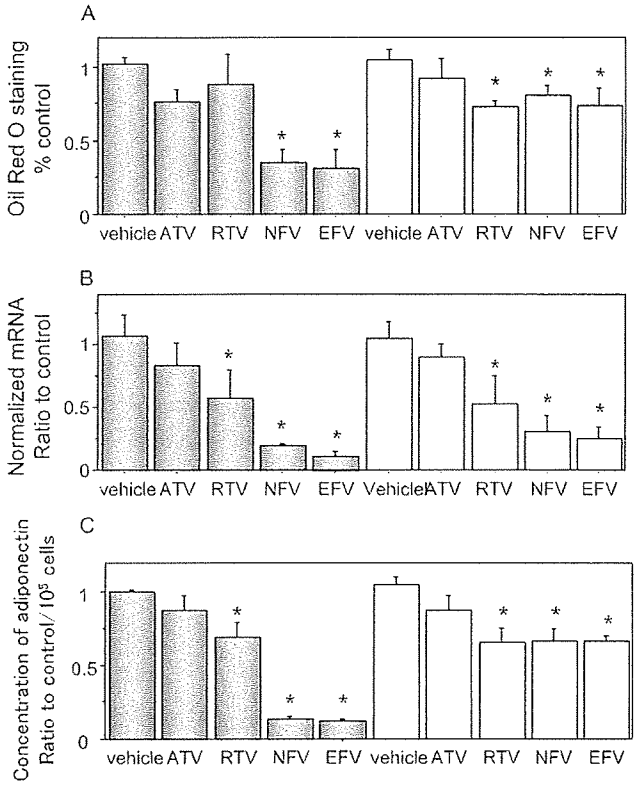

Figure 2. The effect of ARV in pre-adipocytes and mature adipocytes. From confluence (day 0), 3T3-L1 cells were treated with differentiation medium in the absence (control) or in the presence of vehicle or various ARVs. To compare the effects of ARVs on preadipocytes with mature adipocytes, ARVs were added to the medium on day 0, when 3T3-L1 cells are still preadipocytes (gray bar) or on day 6, when 3T3-L1 cells differentiate to mature adipocytes (white bar). (A) The effects of ARVs on triglyceride accumulation during 3T3-L1 adipose conversion. On day 7, the cells were stained with Oil red O. Staining was quantified at 520 nm after solubilization and expressed as %±SE of the control on day 7. (B) The effect of ARV on adiponectin mRNA levels in 3T3-L1 cells. On day 7, total RNA was prepared and mRNA levels were determined by real time RT-PCR. The results shown are after correction for the levels of β actin mRNA and normalized to the controls and represent the mean±SE. (C) The effect of ARV on adiponectin secretion in 3T3-L1 cells. At day 7, each supernatant was collected. Then, concentrations of the adiponectin were determined using an ELISA. Results shown are normalized to the controls and represent the mean±SE. Significance of difference between vehicles and other ARVs was evaluated by using the Dunnett test. EFV: efavirenz, RTV: ritonavir, ATV: atazanavir, NFV: nelfinavire

on the expression of adiponectin mRNA. In 3T3-L1 cells, the expression of adiponectin mRNA was decreased by RTV, NFV and EFV, but not by ATV. These data are consistent with our *in vivo* data. The pre-mature adipocytes were more sensitive to the effect of NFV and EFV on adiponectin mRNA and lipid accumulation than mature adipocytes. Since adiponectin is mainly produced from mature adipocytes, some factors, such as the regulatory mechanisms for the differentiation of adipocytes, might be involved in the expression of adiponectin. In fact, in subcutaneous fat from individuals from HIV-associated lipodystrophy, decreased expression of some differentiation-associated gene, such as sterol regulatory element binding protein 1, CAAT enhancer binding protein α, and peroxisome proliferators-activated receptor-γ have been described (16). On the other hand, the effects of RTV and ATV on the expression of adiponectin mRNA and lipid accumulation were not significantly different between pre-mature adipocytes and mature adipocytes. Further, the effects of RTV, NFV, and EFV on adiponectin expression were observed also in mature adipocytes, though to a lesser extent than in NFV- and EFV-treated pre-mature adipocytes. These results showed some mechanisms other than differentiation-associated gene might be involved in the expression of adiponectin. Adipose cells are highly sensitive to oxidative stress, and it has been reported that oxidative stress is one of the mechanisms that regulates adiponectin expression. Using a reporter construct containing the adiponectin promoter, reactive oxygen species (ROS) have been shown to reduce the transcriptional activity of the adiponectin gene in 3T3-L1 adipocytes (17). From the current data, it is certain that various mechanisms are involved in the regulation of adipokine expression and that the effects of ARVs on adipogenesis and adiponectin expression may vary among different drugs. The distinct metabolic effect of ARVs could therefore be a consequence of their differential effects on both the production of adiponectin and the adipocyte physiology.

We have shown that ATV, in comparison to RTV, NFV and EFV, causes less inhibition of adiponectin secretion and lipid accumulation. Furthermore, the replacement of RTV, NFV, and EFV to ATV did not decrease the serum HMW adiponectin level and ATV replacing therapy has been associated with a decrease in hyperlipidemia and an increase in serum HMW adiponectin in HIV-infected patients. In the same way, ATV leads to a reversal in the impairment of adiponectin secretion or other metabolic abnormalities in 3T3-L1 cells. These properties could underlie the favorable metabolic side effect profile of ATV observed in its clinical use.

This study showed the direct effect of ARV on the lipid metabolism, but it is possible that such abnormalities in adiponectin and lipid metabolism in HIV-infected individuals are the result of either the consequence of HIV infection itself or of cytokine/chemokine released from infiltrating macrophages, or several other factors.

This study provides important new information for clinicians and patients regarding the relative risk and benefits of available antiretroviral regimens for the initial therapy of HIV-1 infection. EFV and some PIs except for ATV containing ART decreased serum HMW-adiponectin, which is associated with dyslipidemia and lipodystrophy. Some ARVs, with the exception for ATV decreased the expression of adiponectin in adipocytes *in vitro* and the phenomenon seems

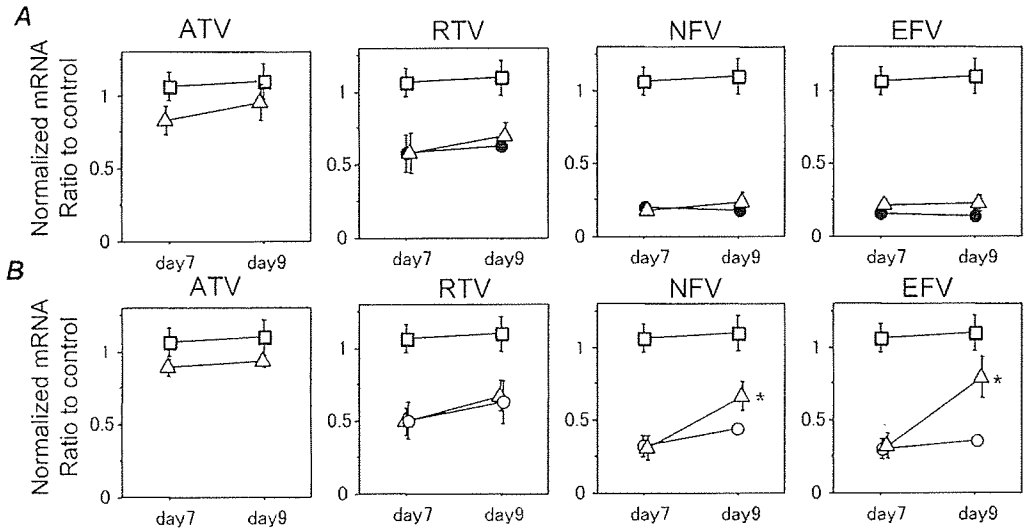

Figure 3. The effect of replacing ARVs with ATV on adiponectin mRNA levels in 3T3-L1 cells. From confluence (day 0), 3T3-L1 cells were treated with differentiation medium in the absence (control) or in the presence of vehicle (□) or various ARVs, which were added to the medium at day 0, when 3T3-L1 cells are still preadipocytes, (●)(A), or on day 6, when 3T3-L1 cells differentiate to mature adipocytes (○) (B). On day 7, the cells were washed and then treated with the medium containing ATV(△) or with the medium with the same ARV as used until day 7 (●, ○). On days 7 and 9, total RNA was prepared and mRNA levels were determined by real time RT-PCR. Results shown are after correction for the levels of β actin mRNA and normalized to the control and represent the mean ± SE. P values are evaluated by Student's t-test. *$p<0.01$ versus the same ARV as used until day 7. EFV: efavirenz, RTV: ritonavir, ATV: atazanavir, NFV: nelfinavir

to be caused by several different mechanisms. A greater understanding of the mechanisms underlying the development of this metabolic effect could lead to safer ARVs, and at the same time lead to the most appropriate treatment for these metabolic side effects of ARVs.

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References

1. Hara K, Horikoshi M, Yamauchi T, et al. Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care **29**: 1357-1362, 2006.
2. Waki H, Yamauchi T, Kamon J, et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem **278**: 40352-40363, 2003.
3. Wand H, Calmy A, Carey DL, et al; INITIO Trial International Coordinating Committee. Metabolic syndrome, cardiovascular disease and type 2 diabetes mellitus after initiation of antiretroviral therapy in HIV infection. AIDS **21**: 2445-2453, 2007.
4. Wohl D, Scherzer R, Heymsfield S, et al; FRAM Study Investigators. The associations of regional adipose tissue with lipid and lipoprotein levels in HIV-infected men. J Acquir Immune Defic Syndr **48**: 44-52, 2008.
5. Bonfanti P, Giannattasio C, Ricci E, et al. HIV and metabolic syndrome: a comparison with the general population. J Acquir Immune Defic Syndr **45**: 426-431, 2007.
6. Nolan D, Mallal S. Complications associated with NRTI therapy: update on clinical features and possible pathogenic mechanisms. Antivir Ther **9**: 849-863, 2004.
7. Lindegaard B, Keller P, Bruunsgaard H, Gerstoft J, Pedersen BK. Low plasma level of adiponectin is associated with stavudine treatment and lipodystrophy in HIV-infected patients. Clin Exp Immunol **135**: 273-279, 2004.
8. Samaras K, Wand H, Law M, Emery S, Cooper D, Carr A. Prevalence of metabolic syndrome in HIV-infected patients receiving highly active antiretroviral therapy using International Diabetes Foundation and Adult Treatment Panel III criteria: associations with insulin resistance, disturbed body fat compartmentalization, elevated C-reactive protein, and [corrected] hypoadiponectinemia. Diabetes Care **30**: 113-119, 2007 (Erratum in: Diabetes Care **30**: 455, 2007).
9. Möbius U, Lubach-Ruitman M, Castro-Frenzel B, et al. Switching to atazanavir improves metabolic disorders in antiretroviral-experienced patients with severe hyperlipidemia. J Acquir Immune Defic Syndr **39**: 174-180, 2005.

10. Kinlaw WB, Marsh B. Adiponectin and HIV-lipodystrophy: taking HAART. Endocrinology **145**: 484-486, 2004.
11. Seoane E, Resino S, Micheloud D, et al. Lipid and apoprotein profile in HIV-1-infected patients after CD4-guided treatment interruption. J Acquir Immune Defic Syndr **48**: 455-459, 2008.
12. Hara K, Boutin P, Mori Y, et al. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes **51**: 536-540, 2002 (Erratum in: Diabetes **51**: 1294, 2002).
13. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun **257**: 79-83, 1999.
14. Yang WS, Lee WJ, Funahashi T, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab **86**: 3815-3819, 2001 (Erratum in: J Clin Endocrinol Metab **87**: 1626, 2002).
15. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology **145**: 2273-2282, 2004.
16. Chaparro J, Reeds DN, Wen W, et al. Alterations in thigh subcutaneous adipose tissue gene expression in protease inhibitor-based highly active antiretroviral therapy. Metabolism **54**: 561-567, 2005.
17. Furukawa S, Fujita T, Shimabukuro M, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest **114**: 1752-1761, 2004.

症　例

治療後ウエスタンブロット法にて抗HIV抗体が陰性化し持続しているHIV-1感染症の1例

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(平成20年10月28日受付)
(平成21年2月10日受理)

Key words: human immunodeficiency virus (HIV)-1, Western blot

序　言

ウエスタンブロット法（WB法）は，HIV感染の確認検査として広く用いられており，種々のHIV構成タンパクに対する特異抗体を検出する方法である．これらの抗体は，感染早期には産生量は少なく，抗原に対する親和性も低いが，時間の経過とともに抗原の暴露を受けることにより産生量が増加し，親和性も高くなるといわれている．そのため，WB法では通常，感染から時間が経過するに従い，検出される抗体の種類が増えていく．

今回，初診時のWB法にて2種類の抗体が検出されたにも係わらず，その後の経過中に抗体が消失した症例を経験した．抗体消失の機序を解明するため，本症例でのHIV特異的抗原への反応性を評価し，他のHIV陽性患者との比較検討を行った．

症　例

症例：41歳，男性．

主訴：微熱．

既往歴，家族歴：特記事項なし．

現病歴：2004年12月頃より体重減少出現，2005年3月より微熱が認められるようになった．保健所にて抗HIV抗体（PA法，ELISA法）陽性を指摘され当院受診，CD4陽性リンパ球数(CD4)37/μL，HIV-RNA 3.4×10^5コピー/mLであり，胸部CTにてすりガラス陰影を認めたため，精査加療目的にて入院．なお，5年前のHIVスクリーニング検査は陰性であった．数カ月以内にHIV-1感染のリスクはあったが，それ以前にも感染のリスクはあり，正確な感染時期は不明であった．

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入院時現症：身長175cm，体重65kg，体温37.5℃，口腔内白苔は認めない．表在リンパ節触知せず，胸腹部所見異常なし，神経学的異常所見は認めない．

検査所見（Table 1）：末梢血白血球5,000/μL（リンパ球10.3%），CRP 5.9g/dL, CD4陽性T細胞数37/μL，CD4/CD8 0.2，HIV-RNA 3.4×10^5コピー/mL，サブタイプB．抗HIV-1抗体(ELISA法)陽性，Western blot（WB）法gp160，p18にバンドあり．IgG，IgA，IgM，IgGサブクラス異常なし．β-D-グルカン42.6pg/mL，喀痰PCR *Pneumocystis jiroveci* 陽性，抗酸菌陰性．胸部CTにてすりガラス陰影を認める．

臨床経過（Fig. 1）：入院後，PCPの治療を行ったが，治療薬であるアトバコンに対しアナフィラキシー反応を示したため28病日にHydrocortisone　100mgを使用した．その後，喀痰より結核菌が検出されたため58病日より抗結核剤の内服を開始．76病日から抗HIV薬の内服を開始した．93病日より発熱を認め，薬剤アレルギーおよび免疫再構築症候群の可能性を考えprednisoloneを使用した（20mg/日×7日間，10mg/日×7日間）．その後，発熱は改善，喀痰からの結核菌は消失，胸部CTの所見も改善したため退院となった．抗HIV薬開始後，HIV-RNA量は徐々に低下し，6カ月後には感度（50コピー/mL）以下となった．以後，ウイルス量は感度以下で経過している．HIV-DNAも同様に徐々に減少し2006年4月には検出感度以下となった．CD4陽性細胞数は徐々に増加し6カ月後に123/μL，現在300～400/μLにて経過している．HIV抗体に関しては，WB法（ラブブロット1；富士レビオ社）にて初診時gp160，p18にバンドが認められていたが，2005年12月以降，バンドは消失し，2年以上経過した現在でもその状態が持続している．PA法によるHIV-1　Ab（ジェネディアHIV-1/2ミックス

Table 1 Laboratory data on admission

CBC		Virology	
WBC	5,000 /μL	Hbs Ag	(−)
Neut	76.5 %	HCV Ab	(−)
Ly	10.3 %	CMV IgG (EIA)	68.3
Mono	7.6 %	CMV IgM	0.43
Eos	5.6 %	CMV/C7HRP	(−)
RBC	5,230,000 /μL	VZV IgG (EIA)	31.6
Hb	15.3 g/dL	HSV IgG (FA)	×24.4
Ht	46.8 %	HHV-6 IgG (FA)	×160
PLT	143,000 /μL	EB EA-DR IgG (FA)	< 10
		EB VCA IgG (FA)	×160
Serology/Immunology		measles (EIA)	35.3
CRP	5.99 g/dL	Infection	
IgG	1,389 mg/dL	β-D glucan	42.6 pg/mL
IgA	454 mg/dL	< PCR analysis of sputum >	
IgM	98 mg/dL	TB	(−)
CD4	37 /μL	MAC	(−)
CD4%	8 %	PCP	(+)
CD8	264 /μL		
CD8%	61 %	HIV-RNA (subtype B)	340,000 copies/mL
CD4/8	0.2		

EIA: Enzyme Immunoassay, FA: fluorescence antibody technique
TB: tuberculosis, MAC: Mycobacterium Avium complex, PCP: Pneumocystis Pneumonia

PA；富士レビオ社），ELISA 法による HIV-1 Ag/Ab（2005 年 3 月，4 月，12 月はジェンスクリーン HIV Ag-Ab；富士レビオ，2007 年 5 月はアーキテクト HIV Ag/AB コンボアッセイ；アボットジャパンにて測定）においても titer が徐々に低下し，2005 年 12 月以降は陰性化した．なお，PA 法に関しては他の測定キット（セロディア HIV（Type 1）：富士レビオ）にて測定を行い，128 倍（2005 年 12 月）であった．

方　法

本症例の HIV-1 抗原に対する反応性を評価し，他の HIV-1 感染者との比較を行った．対象は本症例および当院通院中の HIV-1 陽性者 10 名（ステロイド使用歴あり 4 名，ステロイド未使用かつ抗 HIV 薬投与中 3 名，ステロイド未使用かつ抗 HIV 薬投与中 3 名）（Table 2）．末梢血単核球を分離し Phytohaemagglutinin-P（PHA）（2μM），および HIV-1 Gag p24（1μM；コスモバイオ），HIV-1 Gag p17（0.2μM；コスモバイオ），HIV gp41（0.2μM；Fitzgerald Industries Internal Inc）で刺激，72 時間後に，①リンパ球増殖試験（CellTiter 96 AQueous One Solution Cell proliferation Assay：Promega），②IFN-γ mRNA の定量（real-time RT-PCR 法），③培養上清の IFN-γ の濃度の測定（human IFN-γ ELISA high sensitivity；Bender MedSystems）を行った．測定は，各々の症例につき 2 回ずつ行った．有意差は student t-test にて検定し $p<0.05$ を有意差ありとした．

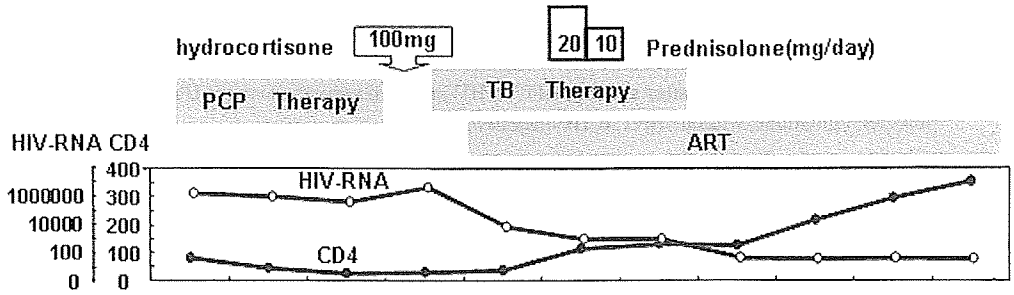
結果（Fig. 2）

本症例の末梢血から分離した単核球は，PHA に対しては，リンパ球刺激試験，IFNγ mRNA 発現量，IFNγ 産生量，いずれにおいても反応を示した．一方，HIV 特異抗原に対する反応は，上記のいずれにおいても HIV 非感染者と同様，反応は認められなかった．他の HIV 感染者においては HIV 特異抗原に対し反応を示した．p24 や p17 に対する反応は，ステロイド投与群や抗 HIV 薬投与群では，未治療者群に比べ低下していた．gp41 に対する反応においても，ステロイド投与群や抗 HIV 薬投与群では，未治療者群に比べ反応が低下する傾向があった．

考　察

HIV 感染症の診断は，血清中の抗 HIV 抗体や HIV 抗原，HIV 遺伝子の検出にて行う．まず，粒子凝集反応（PA 法），ELISA 法などの高感度スクリーニング検査で HIV 抗体，および HIV 抗原/抗体を検出する．スクリーニング検査には偽陽性が約 0.3% 認められるため，陽性の場合は WB 法や HIV-RNA 量の確認検査を行い診断する．WB 法は HIV-1 のコア蛋白（p17，p24，p55），ポリメラーゼ（p31，p51，p66），エンベロープ（gp41，gp120，gp160）に対する抗体を検出し gp120/160 と gp41 もしくは p24 に対する抗体が認められる場合に陽性とする．抗 HIV 抗体が偽陰性になる確率[1]は，0〜2% と調査地域の HIV 感染率や検査対象によっても異なる．Farzadegan H らの薬物使用者（intravenous drug users；IVDUs）を対

Fig. 1　Clinical course.
Peripheral blood HIV-1 RNA load (HIV-RNA), CD4 positive T lymphocyte counts (CD4), HIV-1 Ag/Ab ELISA (ELISA), patterns of Western blot results are shown.
PCP; Pneumocystis pneumonia, TB; Tuberculosis, ART; antiretroviral therapy, ELISA; enzyme-linked immunosorbent assay.



	'05 Mar	Apr		May	Jun		Jul	Dec	'06 Jul	Sep	'07 May
gp160(env)	+	+	+	+	+	+	+	-	-	-	-
gp120(env)	-	-	-	-	-	-	-	-	-	-	-
p68(pol)	-	-	-	-	-	-	-	-	-	-	-
p55(gag)	-	-	-	-	-	-	-	-	-	-	-
p52(pol)	-	-	-	-	-	-	-	-	-	-	-
gp41(env)	-	-	-	-	-	-	-	-	-	-	-
p40(gag)	-	-	-	-	-	-	-	-	-	-	-
p34(pol)	-	-	-	-	-	-	-	-	-	-	-
p24/25(gag)	-	-	-	-	-	-	-	-	-	-	-
p18(gag)	+	+	+	+	+	+	+	-	-	-	-
HIV-Ag/Ab	6.8	5.01	3.68					0.9			0.55
(ELISA)	+	+	+					-			-
HIV-Ab (PA)	512	512						64			
	+	+						+			

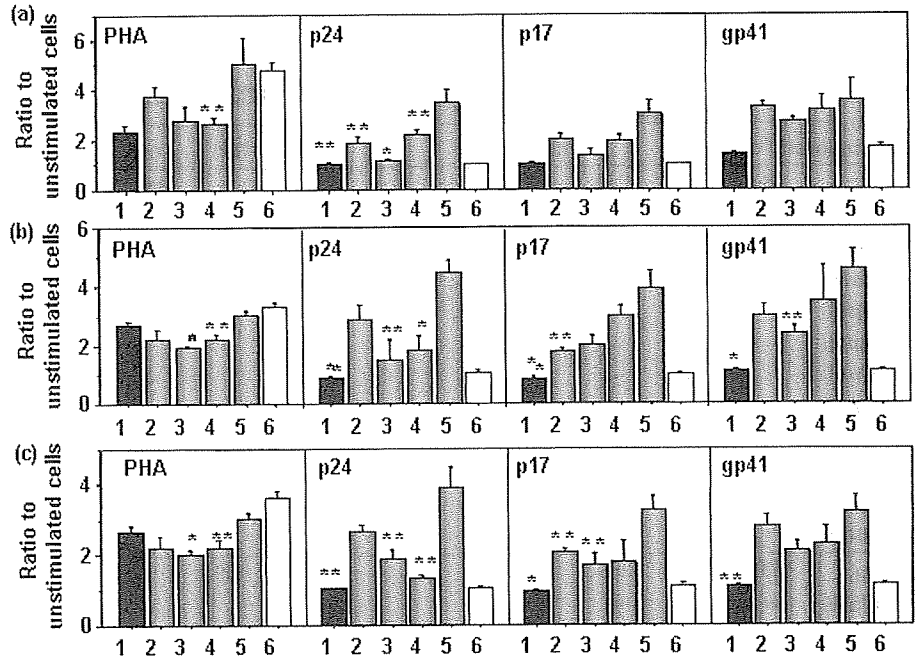
Table 2　Patient profiles

	Age	CD4 (present)	HIV-RNA (present)	ARV Duration (years)	CD4 (min) (/μL)	Steroid use	Steroid dose (max)
Case	41	345	< 50	2.4	7	before ART after ART	Hydrocortisone 100mg PSL 20mg
1	36	809	< 50	2	148	before ART	mPSL 500mg
2	35	874	< 50	1	236	before ART	mPSL 500mg
3	37	231	< 50	2	15	after ART	PSL 20mg
4	49	514	< 50	3.6	4	after ART	PSL 30mg
5	47	595	< 50	3.5	111	—	0
6	50	551	< 50	1	262	—	0
7	30	513	< 50	1.8	243	—	0
8	39	455	13,000	0	455	—	0
9	29	498	4,300	0	245	—	0
10	30	325	8,400	0	224	—	0

CD4 (min): minimum CD4 T lymphocyte counts during clinical course,
Steroid dose (max): maximum steroid dose use during clinical course,

象とした研究では 0.3%[2]，Gibbonns Jらの血友病を対象とした研究では 1.8% と報告されている[3]．偽陰性の原因としては，(1) ウインドウ期，(2) 抗体の消失：進行した免疫不全状態や，急性期に抗 HIV 剤を開始した場合など，(3)無γグロブリン血症，(4)HIV-2 感染，(5) ウイルス側の要因，(6) 検査技術の問題，(7) 原因不明，などが挙げられる．本症例の場合，感染が判明してから 2 年以上が経過しており，(1) のウ

Fig. 2 Response of the peripheral blood mononuclear cells (PBMC) to HIV-specific protein. 5×10^4 PBMCs were incubated in triplicate with PHA (2μM), Gag p24 (1μM), Gag p17 (0.2μM), gp41 (0.2μM) for 3 days. (a) Proliferation assays were performed with a Cell proliferation Assay kit. (b) IFNγ mRNA expression of PBMC was evaluated by quantitative RT-PCR. (c) Secretion of IFNγ to the culture medium was assayed using an ELISA. Results are expressed as the ratio of data of PBMC with antigens to data of PBMC without antigens. * $p < 0.01$, ** $p < 0.05$ vs. patients without antiretroviral therapy (Student *t*-test).
1. case, 2. HIV-infected individuals with steroid use before starting ART, 3. HIV-infected individuals with steroid use after starting ART, 4. HIV-infected individuals with ART, 5. HIV-infected individuals without ART, 6. HIV-negative controls.



インドウ期とは考えにくい．またIgGは正常範囲であり，麻疹やヘルペスウイルス属に対する抗体は検出されているため，(3) の無γグロブリン血症も否定的である．HIV-1のRNAはPCRで検出されており，HIV-2に対する抗体はWB法にて陰性であった．検査は再検にて確認されており，またPA法やELISA法によるHIV抗体は，他の測定キットでも同様の結果であったため，技術的な問題も考えにくい．ウイルス側の要因としては，HIV-1のHLA-IもしくはHLA-II拘束性のエピトープが変異することによりHIVがT細胞からの認識を妨げている場合があるという報告がある[4]．本症例の場合，*in vitro*にてリコンビナントのp24，p17，gp41に対する反応がいずれも低下しており，ウイルス側の要因ではなく，宿主側の要因が主体であると考えられる．以上より，本症例では，何らかの要因によりHIVに対する抗体が消失したと考えられる．実際，初診時に認められたgp160，p18に対する抗体は，6カ月後には，消失している．Fig. 2に示すように，本症例はp24，p17，gp41に対する反応が消失していた．他の症例は，いずれもHIV抗原に対する反応性は保たれていたが，ステロイド投与群，抗HIV剤投与群では，未治療者に比べ，反応性が低下していた．ステロイドの使用によりHIV特異的CTLが抑制されHIV-1タンパクに対する反応性が低下したという報告がある[5]．また抗HIV薬の影響については，抗HIV薬そのものが，免疫系に影響を与えるという報告[6]，および抗HIV薬にて抗原刺激（体内のHIV）が減少するためにHIV特異的CTLやHIVに対する抗体の産生が抑制されるという報告がある[7]．後者に関連して，急性感染時期の抗HIV薬導入により，抗体の陽転化が遅延したという報告[8][9]や，垂直感染時の早期の抗HIV薬導入によりHIV特異的CTLが消失した症例の報告がある[10][11]．本症例では，感染時期の同定は出来ないが，HIV-1への反応が不十分な時期にステロイドの使用および抗HIV薬の導入を行い，抗原刺激の減少（HIV-RNA，HIV-DNAの減少）とともに，HIV-1に反応するリンパ球のクローンが消失した可能性がある．

今回，我々は抗HIV抗体がWB法による判定で長期間陰性で，HIV抗原に対する*in vitro*の反応も消失しているHIV-1感染症の症例を経験した．今回の症例はHIVに対する免疫機構を考える上で興味深い症

例と考えられる．また，本症例は，末梢血中の HIV-1 RNA が検出感度以下であり，抗 HIV-1 抗体も検出されないことから，標準的な検査では，HIV-1 感染陰性と判定され得るため注意が必要である．

謝辞：抗 HIV 抗体測定にご協力いただきました富士レビオ社に深謝いたします．また，「HIV-1 抗原に対する反応性」の実験にご協力いただきました高橋真梨子氏に深謝いたします．

文　献

1) Van de Perre P, Simonon A, Msellati P, Hitimana DG, Vaira D, Bazubagira A, *et al.*：Postnatal transmission of human immunodeficiency virus type 1 from mother to infant. A prospective cohort study in Kigali, Rwanda. N Engl J Med 1991；325：593—8.
2) Farzadegan H, Vlahov D, Solomon L, Muñoz A, Astemborski J, Taylor E, *et al.*：Detection of human immunodeficiency virus type 1 infection by polymerase chain reaction in a cohort of seronegative intravenous drug users. J Infect Dis 1993；168：327—31.
3) Gibbons J, Cory JM, Hewlett IK, Epstein JS, Eyster ME：Silent infections with human immunodeficiency virus type 1 are highly unlikely in multitransfused seronegative hemophiliacs. Blood 1990；76：1924—6.
4) Harcourt GC, Garrard S, Davenport MP, Edwards A, Phillips RE：HIV-1 variation diminishes CD4 T lymphocyte recognition. J Exp Med 1998；188：1785—93.
5) Harrer T, Bäuerle M, Bergmann S, Eismann K, Harrer EG：Inhibition of HIV-1-specific T-cells and increase of viral load during immunosuppressive treatment in an HIV-1 infected patient with Chlamydia trachomatis induced arthritis. J Clin Virol 2005；34：224—30.
6) Angel JB, Parato KG, Kumar A, Kravcik S, Badley AD, Fex C, *et al.*：Progressive human immunodeficiency virus-specific immune recovery with prolonged viral suppression. J Infect Dis 2001；183：546—54.
7) Pitcher CJ, Quittner C, Peterson DM, Connors M, Koup RA, Maino VC, *et al.*：HIV-1-specific CD4＋ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat Med 1999；5：518—25.
8) Selleri M, Orchi N, Zaniratti MS, Bellagamba R, Corpolongo A, Angeletti C, *et al.*：Effective highly active antiretroviral therapy in patients with primary HIV-1 infection prevents the evolution of the avidity of HIV-1-specific antibodies. J Acquir Immune Defic Syndr 2007；46：145—50.
9) Rosenberg ES, Altfeld M, Poon SH, Phillips MN, Wilkes BM, Eldridge R, *et al.*：Immune control of HIV-1 after early treatment of acute infection. Nature 2000；407：523—6.
10) Luzuriaga K, McManus M, Catalina M, Mayack S, Sharkey M, Stevenson M, *et al.*：Early therapy of vertical human immunodeficiency virus type 1 (HIV-1) infection：control of viral replication and absence of persistent HIV-1-specific immune responses. J Virol 2000；74：6984—91.
11) Desai N, Mathur M, Abu-Lawi K：HIV-1 seronegativity in a child with proved perinatal HIV infection on HAART. Sex Transm Infect 2005；81：377—9.

A Case of HIV-1 Infection that Showed Western Blot Analysis for HIV-1 Negative After Antiretroviral Therapy

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Western blot (WB) is the most widely accepted confirmatory assay for detecting antibodies to the human immunodeficiency virus 1 (HIV-1). We report the case of an HIV-1 patient whose WB was negative for over two years.

A 41-year-old Japanese man with Pneumocystis pneumonia (PCP) and pulmonary tuberculosis referred in March 2005 was found to have positive HIV-1 ELISA and HIV RNA PCR, but HIV-1 WB with only two bands, at gp160 and p18, and no WB HIV-2 band. The CD4 count was 37/μL, and total immunoglobulin, IgG, IgM, and IgG subclasses were normal. The man was treated for PCP and pulmonary tuberculosis, then underwent antiretroviral therapy. He had taken short-terms steroids to treat a drug allergy and immune reconstitution syndrome. Six months later, his serological ELISA tests for HIV-1 and HIV DNA PCR were negative and WB showed no positive band. The CD4 count recovered gradually, and exceeded 350/μL two years later, but WB remained negative. Lymphoproliferative assays and interferon γ expression against HIV-p17, p24, and p41 were studied and compared to those of other HIV-1 infected patients. Our patient showed no response to p17 or p24 and only a weak response to p41. Other patients showed a response to HIV-antigens, but patients with antiretroviral therapy or with histories of steroid use responded more weakly than those with neither. These findings show that HIV-specific lymphocytes decline with antiretroviral therapy and steroid treatment within early HIV infection. It is therefore important to interpret negative serological tests carefully in patients such as ours.

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〈調査報告〉

HIV/AIDS患者に対する医療ソーシャルワーカーの地域連携行動

田　中　千枝子
本　名　　靖

（要旨）

医療財資源の効率化を目的にした，第五次医療法改正の目玉である4疾患5事業制度が始まった．各疾患や事業ごとの地域連携を促進する仕組みである．それに先駆けて，HIV/AIDS医療体制でも整備事業が行われている．これはHIV/AIDSに関するブロック・中核の各拠点病院間また拠点・非拠点病院間の地域医療連携体制づくりの試みである．そこでは医療ソーシャルワーカーには，ミクロレベルの個別事例の直接支援のための連携のみならず，組織や地域に介入するメゾからマクロレベルの連携行動が必要とされる．しかし従来病院に所属している医療ソーシャルワーカーはミクロレベルへの直接介入のサービスマネジメントにとどまり，組織や地域に展開するメゾ・マクロレベルの連携行動としてのソーシャルワークを，通常業務として行っているとは言い難い状況にあるのではないかと考えられた．

そこでHIV/AIDSに対する医療ソーシャルワーカーの地域連携活動についての認識とその実態について，全国の全拠点病院（368名），非拠点病院（800名）のソーシャルワーカーに対して量的調査を平成20年12月から21年1月にかけておこなった．回収率は前者50.8%で，後者で43.8%であった．調査の結果，回答を行った拠点病院の75%のソーシャルワーカーはHIV/-AIDS事例体験を有しており，非拠点病院では15%であった．対象の集団は，専門職団体である日本医療社会事業協会会員とほぼ同じ基本属性を持つ集団であり，性別で8割弱が女性，8割弱が社会福祉士資格をもち，拠点病院ではさらに精神保健福祉士や介護支援専門員資格も3〜4割程度と有意に多く持っていた．しかし拠点病院のソーシャルワーカーの経験年数は非拠点病院よりも有意に少なく，比較的若年層が多かった．

HIV/AIDS拠点および有事例者集団の連携行動の特徴は，メゾのチーム・組織レベルでの認知や理解は得られており，また経験を積んでいることによって，外部との連絡を自分なりに吟味して動き出すという専門職としての自律性を有していることが分かった．また事例経験のないソーシャルワーカーほど，他組織との連携の必要を強調しているが，経験を積めば積むほど，拠点病院として他のスタッフや組織的な認知が深まっていると思われる状況では，むやみに他と連携す