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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-

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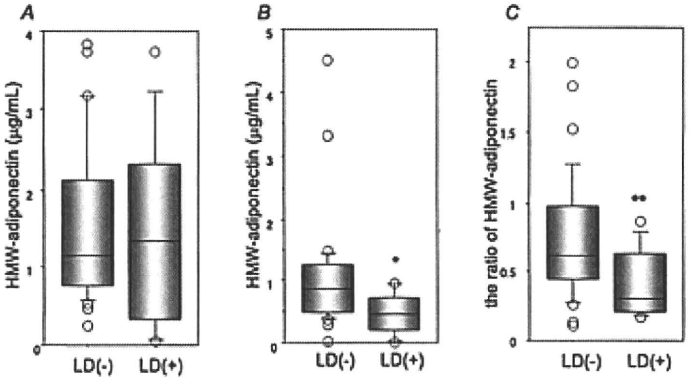

Figure 1. The levels of serum HMW-adiponectin in HIV-infected individuals with/without lipodystrophy. (A) HMW-adiponectin before starting ART, (B) HMW-adiponectin after starting ART, (C) the ratio of HMW-adiponectin after starting ART to that before starting ART. The medians are indicated with horizontal 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

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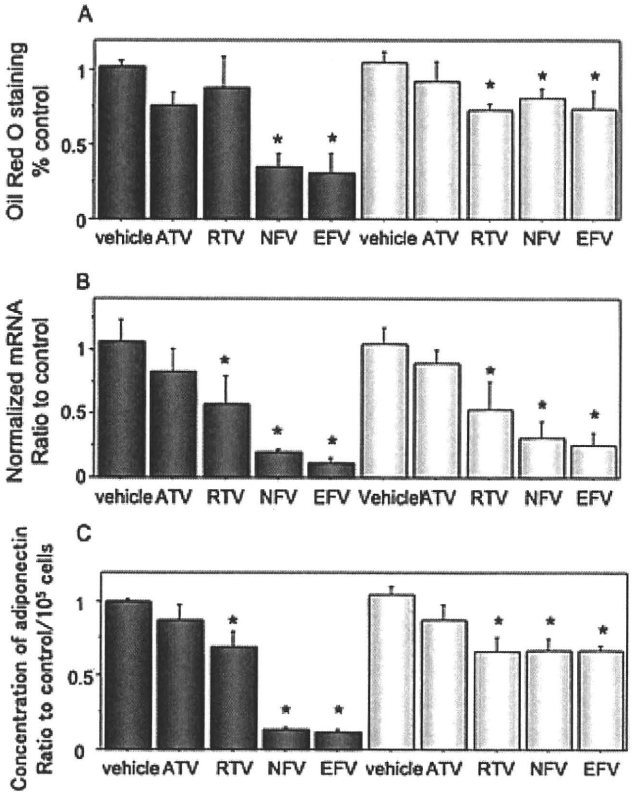

Figure 2. The effect of ARV in pre-adipocytes and mature adipocytes. From confluence (day 0), 3T3-L1 cells were treated with differentiation medium in the absence (control) or in the presence of vehicle or various ARVs. To compare the effects of ARVs on preadipocytes with mature adipocytes, ARVs were 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

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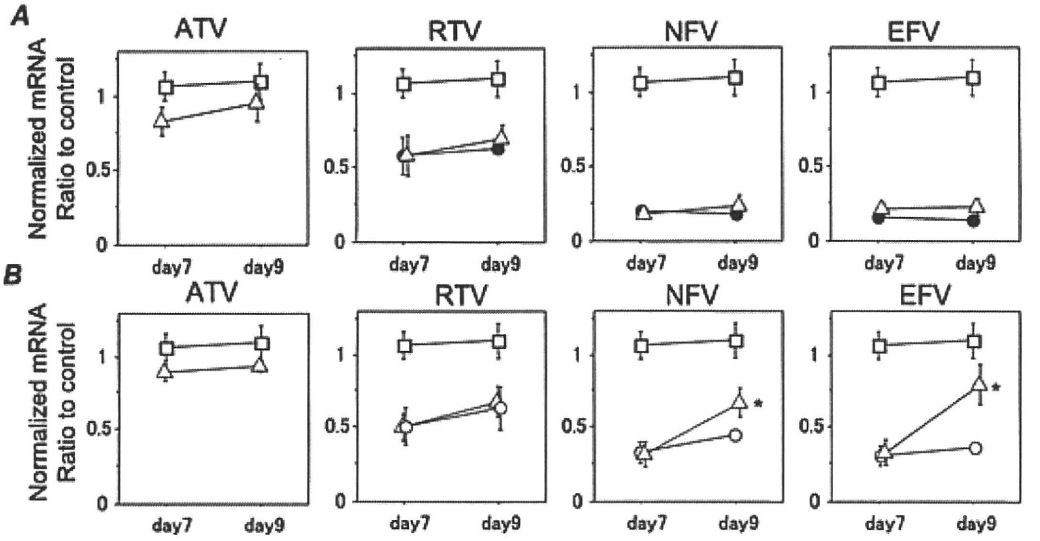

Figure 3. The effect of replacing ARVs with ATV on adiponectin mRNA levels in 3T3-L1 cells. From confluence (day 0), 3T3-L1 cells were treated with differentiation medium in the absence (control) or in the presence of vehicle (□) or various ARVs, which were added to the medium at day 0, when 3T3-L1 cells 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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ORIGINAL ARTICLE

Comparison of the influence of four classes of HIV antiretrovirals on adipogenic differentiation: the minimal effect of raltegravir and atazanavir

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Abstract Antiretroviral therapy for HIV infection is associated with lipodystrophy. However, raltegravir (RAL), a new integrase inhibitor, and atazanavir (ATV), a new generation of protease inhibitor (PI), have not been reported to significantly induce metabolic abnormalities in some clinical studies. The aim of this study was to investigate the influence and molecular mechanisms of RAL and compared it with the other three classes of ARVs (nucleoside reverse-transcriptase inhibitors; NRTI, nonnucleoside reverse-transcriptase inhibitor; NNRTI, and PI) on adipogenesis using 3T3-L1 cells. RAL and ATV had minimal effects on the lipid metabolism of 3T3-L1 cells. NRTI induced a moderate change, and NNRTI and some PIs induced a severe reduction in cell lipid content. These ARVs induced a decrease in the expression of genes associated with lipogenic transcription factors (sterol regulatory-element-binding protein-1c, CAAT box enhancer-binding protein-α, and peroxisome proliferator-activated receptor-γ). The differentiated 3T3-L1 cells were less sensitive to ARV-induced metabolic disturbance than were predifferentiated cells. RAL and ATV did not significantly affect the lipid metabolism in our in vitro study. The other ARVs had a direct influence on adipocytes. Degree and underlying mechanisms of metabolic disturbance differed among different ARVs. These data suggest that the distinct metabolic side-effect profiles of ARVs are a consequences of their differential effects on the adipocyte physiology. A better understanding of the mechanism of ARV-induced metabolic abnormalities could lead to safer use of ARVs or selection of alternative agents for further clinical development.

Keywords HIV-1 · Antiretrovirals · Adipocyte · Lipodystrophy

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Introduction

Highly active antiretroviral therapy (HAART) has helped to control HIV infections and the development of AIDS. However, this antiretroviral therapy (ART) is often associated with severe lipodystrophy, such as peripheral lipoatrophy, central fat accumulation, and hyperlipidemia, but the use of new-generation protease inhibitors (PIs) atazanavir (ATV), and the integrase inhibitor raltegravir (RAL), has been reported to be associated with a decrease in hyperlipidemia [1] and reversal of lipodystrophy [2]. The cellular and molecular mechanisms underlying the metabolic abnormalities induced by ART are unclear, but many studies have shown that the pathogenesis of adipose cell dysfunction includes adverse effects on adipocyte differentiation status [3], survival [4], ability to secrete a variety of adipokines [5], mitochondrial function [6], and recovery from oxidative stress [7] induced by antiretrovirals (ARVs). Different ARVs might induce lipid abnormalities to a different degree though distinct mechanisms, and these properties could underlie the metabolic side-effect profile observed from the use of ARVs.

Adipocyte differentiation involves sequential and coordinated action of several transcription factors that regulate expression of adipocyte-specific genes [8]. Following the initial and transient increase in CAAT box enhancer-binding protein (C/EBP)-β and -δ, peroxisome-proliferator-activated receptor (PPAR)-γ, and C/EBP-α promote expression

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of a number of adipose-specific markers, allowing acquisition of an enlarged, rounded shape and progressive accumulation of cytoplasmic triacylglycerol droplets. C/EBP-α is expressed early during adipogenesis and is involved in the induction of PPAR-γ. Coexpression of C/EBP-α and PPAR-γ has synergistic effects on adipogenic conversion, including lipid metabolism, adipokine secretion, and insulin sensitivity. Adipocyte differentiation is also enhanced by sterol regulatory-element-binding protein (SREBP)-1c. SREBP-1c activates PPAR-γ or related adipogenic transcription factors, thus leading to defective adipogenesis and insulin resistance. Therefore, PPAR-γ, C/EBP-α, and SREBP-1c act in concert to induce and maintain the adipocyte phenotype.

Many studies provide evidence that some PIs influence lipid metabolism by inhibiting degradation of adipogenic transcription factors, such as SREBPs [9, 10], C/EBP-α [11], and PPAR-γ [12, 13]. On the other hand, treatment with nucleoside reverse-transcriptase inhibitors (NRTIs) has been reported to affect mitochondrial functions [2, 14] by depleting mitochondrial DNA (mtDNA) and inhibiting transcription. Integrase inhibitor and the newer generation of PIs have been reported to exhibit antiviral efficacy without adverse affects on lipid metabolism [2, 15]. A re-examination of the molecular pharmacology and toxicology of ART, including these new drugs, may help explain the differences in the metabolic profiles observed among ART in clinical use and lead to the discovery of new drugs that will reduce the incidence of lipodystrophy and related metabolic complications in HIV-infected patients receiving HAART. This study first examined the influence of RAL and then compared it with the four classes of ARVs (NRTI, NNRTI, PI, and integrase inhibitor) with regard to lipid metabolism using well-characterized 3T3-L1 adipocytes [16].

Materials and methods

Cells

This study used the 3T3-L1 cell line, which is one of the most widely used and well-characterized models for studying adipocyte differentiation and function. After stimulation, 3T3-L1 preadipocytes show changes in gene expression and acquire adipocyte characteristics, such as a spherical shape and accumulation of triglyceride-rich lipid droplets, as signs of differentiation. The 3T3-L1 cells were purchased from the Japanese Collection of Research Bioresources (Tokyo, Japan) and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Postconfluent cells were induced to differentiate by incubation with 0.5 mM 3-isobutylmethylxanthine and 1 μM dexamethasone for 2 days. This was followed by incubation with 10 μg/ml insulin for 2 days. The cells were then maintained in DMEM with 10% FBS for another 2 days. Antiretroviral drugs were added to the medium before and after the differentiation of 3T3-L1 cells.

Antiretroviral drugs

Zidovudine (AZT) was purchased from Calbiochem-Novabiochem. (California, USA). Abacavir (ABC), stavudine (d4T), didanosine (ddI), lamivudine (3TC), efavirenz (EFV), ritonavir (RTV), and nelfinavir (NFV) were purchased from Toronto Research Chemicals Inc. (Ontario, Canada). Atazanavir (ATV) was provided by Bristol-Myers Squibb Company (New York, USA). Lopinavir (LPV) was provided, by Abbott Laboratories (Illinois, USA). Amprenavir (APV) was provided by Glaxo SmithKline (North Carolina, USA). Raltegravir (RAL) was provided by Merck (New Jersey, USA). Drug stocks in dimethyl sulfoxide (DMSO) were stored at −20°C and diluted into culture media. Vehicle controls received the same final DMSO concentration as all drug-treated incubations (0.1%). The peak serum concentration (C_{max}) of each drug are as follows: AZT 1.07–3.03 μM, ABC 3.4–8.19 μM, d4T 2.14 μM, ddI 6.95 μM, 3TC 7.89–15.6 μM, EFV 14.2–28.8 μM, RTV 0.84–21.9 μM, NFV 5.0–8.6 μM, ATV 4.96–8.38 μM, LPV 8.22–22.2 μM, APV 6.68–16.2 μM, and RAL 10.44–11.15 μM. Cells were treated with 10 μM of ABC, AZT, d4T, ddI, 3TC, and NFV; and with 20 μM of EFV, RTV, ATV, LPV, APV, and RAL.

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. Complementary DNA (cDNA) was generated from the RNA using TAKARA RNA Polymerase Chain Reaction (PCR) kit (TAKARA BIO, Shiga, Japan). Real-time PCR was conducted with LineGene33 (BioFlux, Tokyo, Japan) using SYBR Green Realtime PCR Master Mix (TOYOBO Co, Osaka, Japan). The copy numbers of β-actin were determined in every sample tested as internal control to normalize DNA input. The ratio of the normalized mean value for drug-treated samples was calculated and is indicated in the graphs.

Oil red O staining

The cellular lipid content was assessed by lipid staining with Oil red O. Staining was quantified at 490 nm after solubilization using an Adipogenesis Assay Kit (Chemicon International, California, USA).

Quantitation of 8-OHdG in culture medium

The levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the culture medium were determined using a competitive enzyme-linked immunosorbent assay (ELISA) kit (DNA Damage ELISA Kit; Assay Designs Stressgen, Michigan, USA).

Statistical analysis

Results were reproduced in at least three independent experiments and are presented as mean ± standard error (SE). The Fisher least significant difference (LSD) post hoc test was used for multiple comparisons if analysis of variance (ANOVA) was significant. In all statistical comparisons, a P value of <0.05 was considered to be significant.

Results

Effect of ARVs on lipid content in 3T3-L1 cells

Effects of ARVs on dyslipidemia and lipodystrophy differ among ART regimens, but it is nearly impossible to fully assess the separate effects of each class of drug from the clinical data because patients almost always receive a combination of several classes of ARVs. As a result, in vitro models were used to examine the exact influence of the individual drugs on adipocyte development or metabolism using well-characterized preadipocyte 3T3-LI cells. Lipid accumulation in predifferentiated 3T3-L1 cells was dramatically decreased by NFV and mildly decreased by ABC, AZT, d4T, EFV, LPV, and APV (Fig. 1).

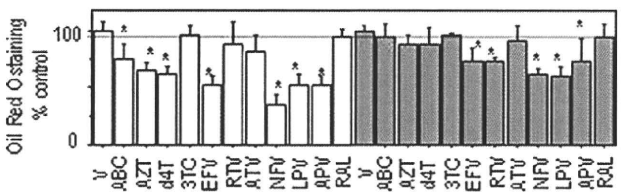

Fig. 1 Effect of antiretrovirals (ARVs) on triglyceride accumulation during 3T3-L1 adipose conversion. From confluence (day 0), 3T3-L1 cells were treated with differentiation medium in the absence (control) or presence of vehicle or various ARVs. To compare the effects of ARVs on preadipocytes with mature adipocytes, ARVs were added to the medium on day 0, when 3T3-L1 cells were still preadipocytes (*white bar*), or on day 6, when 3T3-L1 cells differentiate into mature adipocytes (*gray bar*). On day 7, the cells were stained with Oil red O. Staining was quantified at 490 nm after solubilization and expressed as percent ± standard error (SE) of the control. P values were evaluated by the Fisher least significant difference (LSD) post hoc test. *V* vehicle, *ABC* abacavir, *AZT* zidovudine, *d4T* stavudine, *3TC* lamivudine, *EFV* efavirenz, *RTV* ritonavir, *ATV* atazanavir, *NFV* nelfinavir, *LPV* lopinavir, *APV* amprenavir, *RAL* raltegravir. $*P < 0.01$

It is possible that the effect of ARVs on adipocyte metabolism is different between preadipocytes and differentiated mature adipocytes because the differentiation of adipocytes involves the sequential and coordinated action of several transcription factors that regulate the expression of adipocyte-specific genes and proteins. Therefore, differentiated 3T3-L1 cells were also treated with ARVs and assessed for effects on lipid accumulation. The differentiated 3T3-L1 cells were less sensitive to the effects of ARVs on lipid accumulation level than on immature 3T3-L1 cells. Lipid accumulation in mature 3T3-L1 cells was reduced by EFV, RTV, NFV, LPV, and APV (Fig. 1). On the other hand, RAL had little effect on lipid accumulation in both the immature and mature 3T3-L1 cells.

Effect of ARVs on lipogenic gene expression in 3T3-L1 cells

As shown in Fig. 1, several ARVs reduced lipid accumulation to different degrees. This suggests that various mechanisms are involved in the regulation of lipid accumulation and that different drugs might induce lipid abnormalities through distinct mechanisms. The key lipogenic transcription factors, including SREBP-1c (Fig. 2a), C/EBP-α (Fig. 2b), C/EBP-β (Fig. 2c), and PPAR-γ (Fig. 2d) were investigated to determine the primary cellular mechanisms that underlie these ARV-mediated lipid abnormalities. Treatment with several of the ARVs resulted in marked decreases in the expression of messenger RNAs (mRNAs) for C/EBP-α and SREBP-1c in predifferentiated 3T3-L1 cells (ABC, AZT, d4T, EFV, NFV, LPV, and APV for C/EBP-α; ABC, AZT, d4T, 3TC, EFV, NFV, LPV, and APV for SREBP-1c), and differentiated 3T3-L1 cells (AZT, d4T, EFV, RTV, NFV, LPV, and APV for C/EBP-α; ABC, d4T, 3TC, EFV, NFV, LPV, and APV for SREBP-1c) (Fig. 2). Expression of C/EBP-α and SREBP-1c was not affected by RTV, ATV, or RAL in predifferentiated 3T3-L1 cells. Predifferentiated 3T3-L1 cells were more sensitive to these ARVs than differentiated 3T3-L1 cells, but only RTV had a little stronger effect in the differentiated 3T3-L1 cells than in the predifferentiated cells on C/EBP-α expression. Effects of ARVs on C/EBP-α were similar to those on SREBP-1c. PPAR-γ was also down-regulated by EFV and some PIs (NFV, LPV, APV) but not by NRTIs (ABC, AZT, d4T, 3TC). ATV- and RAL-treated cells remained relatively quiescent. C/EBP-β, which is expressed early during adipogenesis, was not affected by any of the ARVs.

Effect of ARVs on oxidative stress in 3T3-L1 cells

Increased oxidative stress is associated with obesity-related disorders [17], and some ARVs increase oxidative stress in adipocytes in vitro [18] and in vivo [19]. Our study

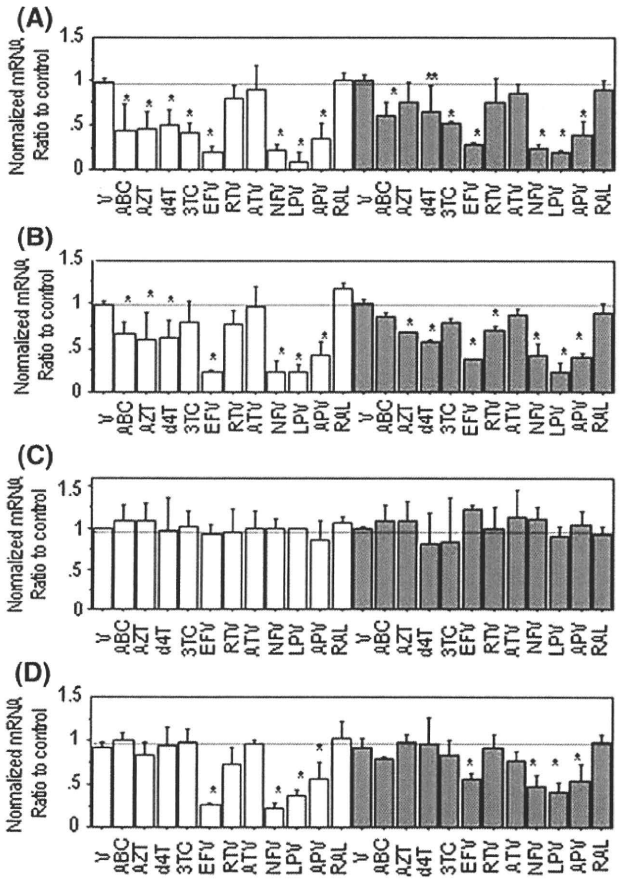

Fig. 2 Effect of antiretrovirals (ARVs) on lipogenic gene expression in 3T3-L1 cells. From confluence (day 0), 3T3-L1 cells were treated with differentiation medium in the absence (control) or presence of vehicle or various ARVs. To compare the effects of ARVs on preadipocytes with mature adipocytes, ARVs were added to the medium on day 0, when 3T3-L1 cells are still preadipocytes (*white bar*), or on day 6, when 3T3-L1 cells differentiate into mature adipocytes (*gray bar*). On day 7, total RNA was prepared, and messenger RNA (mRNA) levels were determined by real-time reverse-transcriptase polymerase chain reaction (RT-PCR). The results shown are after correction for the levels of β-actin mRNA and were normalized to the controls. *P* values were evaluated by Fisher's least significant difference (LSD) post hoc test. **a** sterol regulatory-element-binding protein, **b** CAAT box enhancer-binding protein-α, **c** C/EBP-β, **d** peroxisome-proliferator-activated receptor-γ. *V* vehicle, *ABC* abacavir, *AZT* zidovudine, *d4T* stavudine, *3TC* lamivudine, *EFV* efavirenz, *RTV* ritonavir, *ATV* atazanavir, *NFV* nelfinavir, *LPV* lopinavir, *APV* amprenavir, *RAL* raltegravir. $^{*}P < 0.01$, $^{**}P < 0.05$

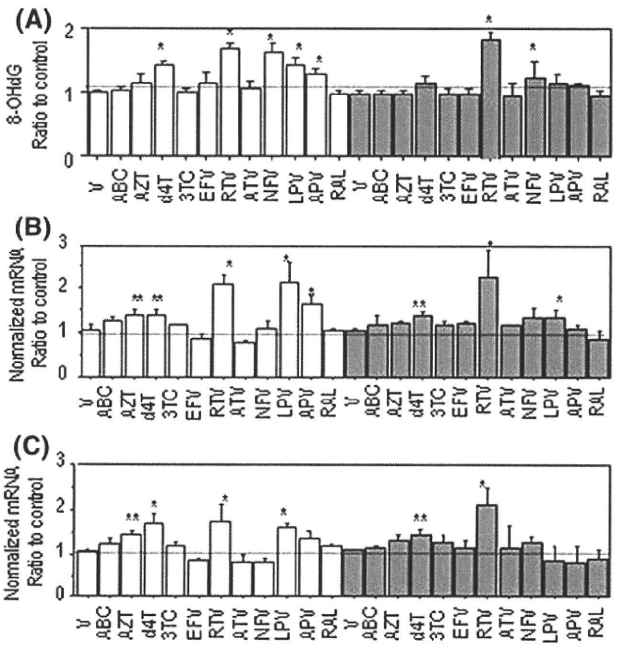

Fig. 3 Effect of antiretrovirals (ARVs) on oxidative stress in 3T3-L1 cells. From confluence (day 0), 3T3-L1 cells were treated with differentiation medium in the absence (control) or presence of vehicle or various ARVs. To compare the effects of ARVs on preadipocytes with mature adipocytes, ARVs were added to the medium on day 0, when 3T3-L1 cells are still preadipocytes (*white bar*), or on day 6, when 3T3-L1 cells differentiate to mature adipocytes (*gray bar*). **a** For the assay of 8-OHdG, each supernatant was collected at day 7. Then, concentrations of 8-OHdG were determined using an enzyme-linked immunosorbent assay (ELISA). Results shown were normalized to the controls and represent the mean ± standard error (SE). On day 7, total RNA was prepared, and messenger RNA (mRNA) levels were determined by real-time reverse-transcriptase polymerase chain reaction (RT-PCR). Effects of the drugs on **b** superoxide dismutase (SOD) and **c** catalase (CAT) were also examined. The results shown were obtained after correction for the levels of β-actin mRNA and were also normalized to the controls. Data are presented as the mean ± standard error (SE). *P* values were evaluated by the post hoc test. *V* vehicle, *ABC* abacavir, *AZT* zidovudine, *d4T* stavudine, *3TC* lamivudine, *EFV* efavirenz, *RTV* ritonavir, *ATV* atazanavir, *NFV* nelfinavir, *LPV* lopinavir, *APV* amprenavir, *RAL* raltegravir. $^{*}P < 0.01$

investigated the influence of the four classes of ARVs on the production of oxidative stress by measuring 8-OHdG in the culture medium. This compound is a modified nucleoside base, which is associated with reactive oxygen species (ROS) and used as a biomarker of oxidative stress. The level of 8-OHdG was increased in the culture medium of predifferentiated 3T3-L1 cells by d4T, RTV, NFV, LPV, and APV and was increased in differentiated 3T3-L1 cells by RTV and NFV (Fig. 3a). Expression of antioxidant enzymes, including superoxide dismutase (SOD, Fig. 3b) and catalase (CAT, Fig. 3c) was also investigated. Generally, oxidative stress, such as that induced by the free-radical superoxide, stimulates the expression of SOD and CAT. Expression of SOD mRNA was increased by the addition of AZT, d4T, RTV, LPV, and APV in predifferentiated 3T3-L1 cells; and d4T, RTV, and LPV in differentiated 3T3-L1 cells. Expression of CAT mRNA was increased by the addition of AZT, d4T, RTV, and LPV in predifferentiated 3T3-L1 cells and of d4T and RTV in differentiated 3T3-L1 cells. Neither SOD mRNA nor CAT mRNA was increased by the addition of ABC, 3TC, EFV, ATV, NFV, or RAL in predifferentiated and differentiated cells. These results and previous studies indicate that the

influence of ARVs on oxidative stress or the antioxidant system differs based upon the class and particular structure of the ARV.

Discussion

ART has been involved in the emergence of a metabolic disorder with potentially severe consequences, but the use of new-generation PIs ATV and the integrase inhibitor RAL has been reported to be associated with a decrease in hyperlipidemia [1] and a reversal of lipodystrophy [2]. Most previous studies examined one or a few classes of ARVs, and each study used different cell systems. Therefore, it is difficult to compare the effects of each drug. Our study investigated the effect of four classes of ARVs (NRTIs, NNRTI, PIs, and integrase inhibitor) using pre- and post-differentiated 3T3-L1 cells. We were especially interested in determining the effects of RAL and ATV compared with other ARVs in order to obtain a better understanding of the molecular basis for the more favorable metabolic side-effect profile associated with RAL and ATV. This is the first study to investigate the effects of RAL on the cellular and molecular regulation of adipocytes.

Expression of C/EBP-α, PPAR-γ, and SREBP-1c were strongly inhibited by some PIs, mildly inhibited by NNRTI and NRTIs, and minimally affected by ATV, RAL, and RTV. These results and the fact that RTV decreased lipid accumulation and increased oxidative stress in mature 3T3-L1 cells more than in predifferentiated cells suggest that the effect of RTV occurs relatively late in adipocyte differentiation. C/EBP-β, which is expressed during early adipogenesis, was not affected by ARVs, suggesting that molecular targets affected by the antiadipogenic properties of ARVs are probably located downstream of this adipogenic transcription factor. This is consistent with previous reports that some ARVs block adipocyte differentiation by inhibiting the expression of C/EBP-α and PPAR-γ [3, 12, 13] or by impairing SREBP-1 intranuclear localization [9, 20]. These findings indicate that altered functions of PPARγ, C/EBP-α, and SREBP-1c play a role in ARV-related dystrophy.

Increased oxidative stress is also considered to contribute to metabolic abnormalities caused by ARVs. Several reports indicate that ROS production in response to ARVs probably results from increased mitochondrial oxidative stress [7, 14]. The marker of oxidative stress, 8-OHdG, was increased by d4T, RTV, NFV, LPV, and APV, and the antioxidant pathway was impaired by NFV. These results indicate that increased oxidative stress by ARVs is due to activation of ROS production and an impaired antioxidant system. Oxidative stress also contributed to ARV-induced lipogenic abnormalities.

Mature 3T3-L1 cells were less sensitive to the lipid-reducing effect of ARVs than were predifferentiated 3T3-L1 cells, and similar results were obtained with regard to the inhibitory effect of ARVs on the expression of lipogenic transcription factors and some oxidative stress markers. One explanation for the differences between preadipocytes and mature adipocytes is that the more differentiated adipocytes have pathways that could enable cells to escape from ARV-induced blockade of the lipogenic pathway, or they may have a system that inactivates and/or decreases the intracellular level of ARVs.

It is notable that the intracellular events reflect lipoatrophy in the form of depleted adipocyte triglyceride stores, and the extent that each ARV influences the adipocytes was in proportion to that observed in clinical manifestations, although functional links between the molecular mechanism and the observed metabolic alterations are still not fully understood. It is certain that metabolic disorder is affected by numerous other modifiers, including genetic predisposition, diet and lifestyle, and HIV-1 infection. However, some features can be avoided by careful selection of ARVs and thus can be effectively treated.

In conclusion, different ARVs acted through distinct mechanisms to induce disruption of adipocyte differentiation and function to different degrees through distinct mechanisms. Whereas most of ARVs affected lipid accumulation, RAL and ATV had no influence on lipid metabolism in our in vitro study. A greater understanding of the mechanisms underlying the development of this metabolic effect could lead to safer ARVs while indicating the best treatment for these metabolic side effects of ARVs.

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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)	×2+1
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				
IgG	1,389 mg/dL		Infection		
IgA	454 mg/dL		β-D glucan		42.6 pg/mL
IgM	98 mg/dL				
			< PCR analysis of sputum >		
CD4	37 μL		TB		(−)
CD4%	8 %		MAC		(−)
CD8	264 μL		PCP		(−)
CD8%	61 %				
CD4/8	0.2		HIV-RNA (subtype B)		340,000 copies/mL

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．

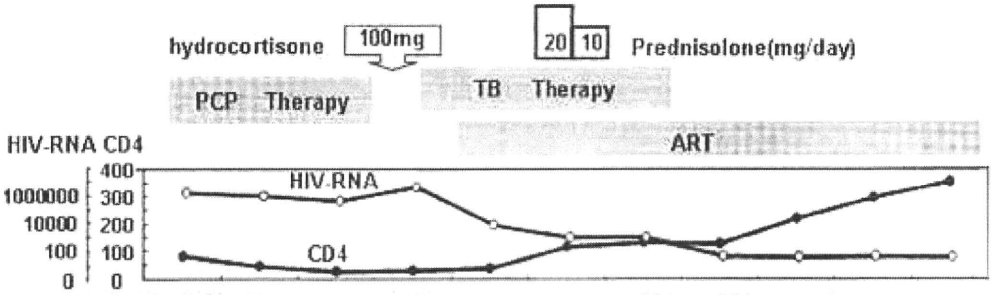
結果（Fig. 2）

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，IFN-γ mRNA 発現量，IFN-γ 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.



HIV-DNA (copies/10^6 cells)				1551			701	391	nd		
	'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			
	+	+						+			

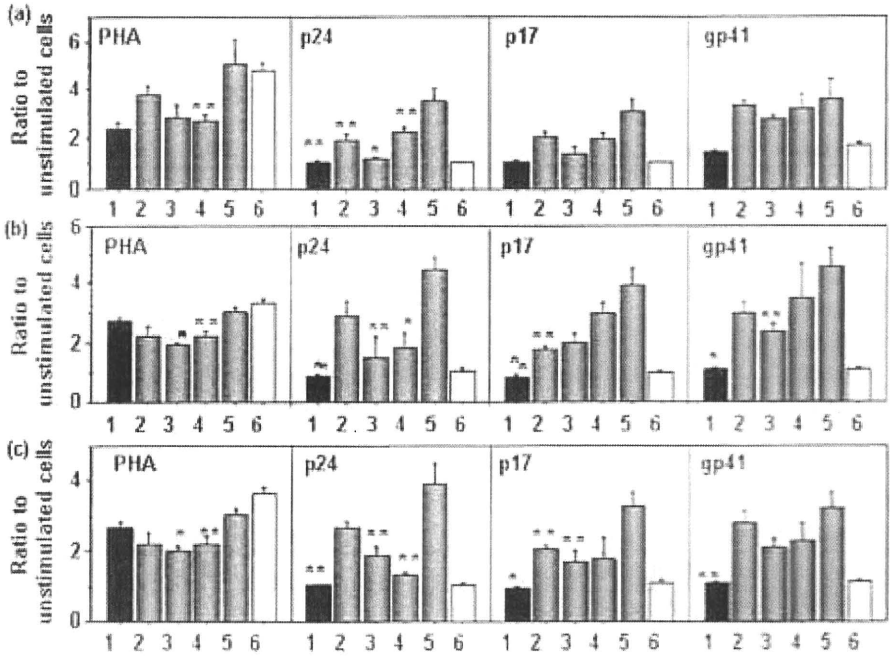
Table 2 Patient profiles

	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	1	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	213	—	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%[3]，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. 5×10^5 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]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[9]．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．

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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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研究分担者　健山　正男

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INTERNAL MEDICINE

□ CASE REPORT □

Pulmonary *Mycobacterium parascrofulaceum* Infection as an Immune Reconstitution Inflammatory Syndrome in an AIDS Patient

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Abstract

Nontuberculous mycobacterial (NTM) infection in HIV (human immunodeficiency virus)-infected patients who have started highly active antiretroviral therapy (HAART) is well known to be one scenario of immune reconstitution inflammatory syndrome (IRIS). We encountered the first case in Japan of an HIV-infected patient with pulmonary *Mycobacterium parascrofulaceum* infection as IRIS. A 34 year-old man with acquired immunodeficiency syndrome (AIDS) was receiving highly active antiretroviral therapy. Lymphadenopathy was observed at the left pulmonary hilum. IRIS was suspected and thoracoscopic surgery was performed to diagnose the cause of lymphadenopathy. Granulomas were observed histologically, and *M. parascrofulaceum* was cultured. This organism was susceptible to Clarithromycin, rifampicin and levofloxacin. After the operation and without treatment, recurrence of *M. parascrofulaceum* infection was not observed. *M. parascrofulaceum* was isolated from several clinical specimens for the first time in 2004. To date, only five cases have been reported.

Key words: *Mycobacterium parascrofulaceum*, immune reconstitution inflammatory syndrome, AIDS

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Introduction

Nontuberculous mycobacterial (NTM) infection in HIV-infected patients who have started highly active antiretroviral therapy (HAART) is well known to be one scenario of immune reconstitution inflammatory syndrome (IRIS). *M. parascrofulaceum* infection was first reported in 2004 (1). There has been little information about *M. parascrofulaceum* infection or therapies for treating this organism. We encountered a case of pulmonary *M. parascrofulaceum* infection in an HIV-infected patient, and describe the clinical features of *M. parascrofulaceum* infection. In addition, since it was possible to culture *M. parascrofulaceum*, the results of the drug sensitivity test are also reported.

Case Report

A 34-year-old man with pneumonia was admitted to the hospital in 2008. He was diagnosed as having *pneumocystis jirovecii* pneumonia (PCP) as well as acquired immunodeficiency syndrome (AIDS). His CD4 count was 9/μL and the HIV-RNA load was very high (128,000 copies/mL) (Table 1). PCP was completely cured by trimethoprim-sulfamethoxazole (TMP-SMX) and prednisolone. Then, TMP-SMX was used for the prophylaxis of PCP.

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Table 1. Laboratory Findings on Admission

Blood cell count		Blood chemistry		Serology	
WBC	3600/mm³	AST	36 IU/L	HBsAg	(-)
CD4	3/mm³	ALT	29 IU/L	HCVAb	0.1COI
RBC	3.98×10^4/mm³	LDH	431 IU/L	HTLV-1	(-)
HGB	12.1 g/dL	T-Bil	0.3 mg/dL	RPR	0.6R.U.
HCT	35.0%	BUN	8.0 mg/dL	TPLA	121T.U.
MCV	88fl	Cre	0.47 mg/dL		
MCH	30.4 pg	Na	139 mEq/L		
MCHC	34.6%	K	4.1 mEq/L	genetics	
PLT	13.1×10^4/mm³	Cl8.6 mEq/L		HIV-RNA	128,000 copies/mL
ESR	82 mm/h	CRP	5.68 mg/dL		
		KL-6	1523 U/mL		
		Beta-D-glucan	147.2 pg/mL		

HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; ESR, erythrocyte sedimentation rate; AST, aspartate aminotransferase; ALT,alanine aminotransferase; LDH, lactate dehydrogenase; T-Bil, total bilirubin; BUN, blood urea nitrogen; Cre, creatinine; CRP, C-reactive protein; HTLV-1, human T cell lymphotropic virus type-1; RPR, rapid plasma reagin test; TPLA, toreponema pallidum latex immunoassay COI, cut off index; R.U., RPRUNITS;T.U.,;TITER UNITS

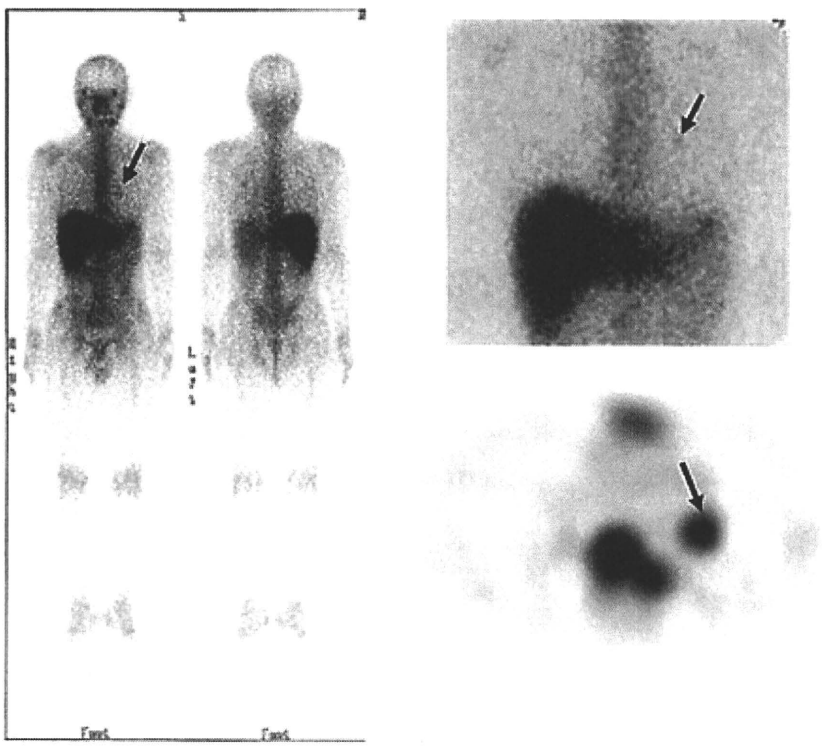

Figure 1. Gallium accumulation at the left hilum (Day 32 after HAART). Arrows: Gallium accumulation at the left hilum.

In January 2009, highly active anti-retroviral therapy (HAART), raltegravir (RAL) + stavudine (d4T) + lamivudine (3TC), was started. HAART was effective and the counts of CD4 increased to 42/μL. The HIV-RNA load decreased to 300 copies/mL in February 2009. Although there were no certain symptoms, IRIS was suspected because the CD4 count was low so a gallium scan to monitor the total body was needed in February 2009. Positive accumulation of gallium was observed at the left hilum (Fig. 1). A chest CT scan revealed lymphadenopathy in the left hilum region where there was a low density area, suggesting a necrotic lesion within it. Then, the size of lymphadenopathy enlarged from 11 mm in diameter to 22 mm. As the cause of lymphadenopathy infection, *M. tuberculosis*, NTM, *Cryptococcus* or *Nocardia* lymphadenopathy was suspected (Fig. 2). HIV-infected patients might develop disseminated disease