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Retrovirus infection and retinoid

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Abstract

Human T-cell leukemia virus type I (HTLV-I) is a human retrovirus and an etiologic agent of adult T-cell leukemia/lymphoma (ATL/ATLL). The subtypes acute and lymphoma have a poor prognosis with a median survival rate of approximately 6 months. Some excellent therapeutic strategies for ATL are required. In present study, we show effects of a new therapeutic agent, retinoid, for proliferation, CD25 expression, NF-κB transcription activity, influence of thiol compound. Furthermore, we clarified a mechanism of retinoid for ATL cells. Finally, we showed an evaluation of retinoid therapy in clinical including for skin involvement.

Key words: retrovirus infection, HTLV-I, HIV, retinoid, anti-retroviral therapy

Introduction

Human T cell leukemia virus type I(HTLV-I) is a human retrovirus that is an etiologic agent of adult T cell leukemia/lymphoma (ATL/ATLL).[1,2] Adult ATL/ATLL is an aggressive lymphoid neoplasm associated with human T-cell leukemia virus type 1 (HTLV-1).[3] ATL, the first human disease found to be associated with retroviral infection, usually occurs in native individuals from HTLV-1 endemic regions, i.e. southern Japan, the Caribbean, intertropical Africa, and Brazil.[4,5] The HTLV-1 provirus is clonally integrated in $CD4^+$, $CD25^+$ activated T lymphocytes, which are leukemic cells characteristic of ATL. The exact mechanism of HTLV-1-induced tumorigenesis has not been fully elucidated, although HTLV-1 infection appears to represent the first event in a multi-step oncogenic process.[6] Diversity in the clinical features of ATL has been noted and four clinical subtypes of ATL have been defined: the acute form, the chronic form, the smoldering form, and the ATL lymphoma type.[7] The acute and lymphoma types of ATL have a poor prognosis with a median survival of about six months.[8] This extremely bad outcome is mainly due to an intrinsic resistance of the leukemic cells to conventional or even high doses of chemotherapy and to a severe immuno-suppression.[8-11] The use of allogenic BMT in the treatment of ATL has been reported, but a high toxicity and transplant-related mortality were observed in immuno-compromised patients.[12-15] A more effective therapy is therefore needed.

Vitamin A and its analogs (retinoid) influence the growth and differentiation of normal and malignant cells, and have been shown to possess anticarcinogenic and antitumor activities in vitro and in vivo.[16,17] Retinoic acid (RA) influences the clonal growth of normal human myeloid cells and induces the differentiation of both HL-60 cells (classified as a celll from a myeloblastic leukemia) and fresh human acute promyelocytic leukemia cells into normal granulocytes.[18-20] It has been reported that RA inhibits the growth of some tumor cells.[21-23] Tax is a specific gene of ATL that immortalizes human T-cells.[24] Tax, a 40 kD protein, is a transcription trans-activator of HTLV-1 that interacts with cellular transcriptional factors to activate HTLV-1 gene expression and HTLV-1 transformation of human T lymphocytes.[24,25] Tax activates HTLV-1 gene expression by increasing the binding of the cyclic AMP-responsive element-binding protein/activating transcription factor (CREB/

ATF) proteins and the coactivator CBP (CREB binding protein) to the three 21-bp repeats in the long terminal repeat of HTLV-1,[26,27] and also activates immediate early genes (c-fos, c-jun, egr-1, and egr-2), a receptor gene (IL-2Rα), and cytokine genes (IL-2, IL-6, TGF-β, GM-CSF).[24,25] Furthermore, tax interacts with the ankyrin motifs in I-κB and NF-κB p105 and dissociates from or interferes with the complex I-κB/NF-κB, which is involved in the transcriptional activation of NF-κB in the cytoplasm.[28] It has also been shown that NF-κB was transported into nuclei and activated to induce the expression of cytokine and receptor genes.[25,25,29] Inhibition of NF-kB activity is related for induction of apoptosis, and thus the Rel/NF-κB family plays important roles in the proliferation and differentiation of various cells in vitro. Already, Mori et al. have reported that NF-κB is constitutively activated in primary ATL cells as well as in the HTLV-1-positive T-cell line TL-Om1 independent of Tax protein.[30] Furthermore, we have suggested that the target molecule of all-*trans* retinoic acid (ATRA) may be tax or some molecule in the tax- NF-κB signal pathway.[31] At the present time, the mechanism of ATRA's effect in ATL cells is not clear. In this article, we showed effects of ATRA in the aspect of 1) growth inhibition and CD25 down-regulation, 2) inhibition of NF-κB transcription, 3) effects of thiol compound, 4) effects for skin involvement, 5) mechanism of ATRA action, 6) clinical application, 7) effects for HIV infection.

Results and Discussion

Growth inhibition and down-regulation IL-2Rα/CD25 by ATRA

We initially assessed the effect of ATRA to HTLV-I positive T cell lines, HUT102 and ATL-2 cells. Incubation of those cells with ATRA for 48 h inhibited [^{3}H]-thymidine incorporation. When those cells were treated with ATRA, as shown in Figure 1a, [^{3}H]-thymidine incorporation was decreased significantly. To assess the effect of ATRA to the cell surface antigen, we observed the expression of IL-2Rα/CD25 by flow cytometry. Incubation of ATL-2 cells for 48 h with 10^{-5} M ATRA for 48 h also resulted in down-regulation of CD25 expression (Figure 1b). Two peaks were apparent on flow cytometric analysis of ATL-2 cells, treated with ATRA, suggesting the existence of sensitive and resistant clones to ATRA. Jurkat and MOLT-4 (Figure 2) were incubated with ATRA for 48 h and assayed for [^{3}H]-thymidine incorporation. However, no growth inhibition was observed on both T cell lines.

The mechanism responsible for the difference in sensitivity of HUT102 cell clones to RA with

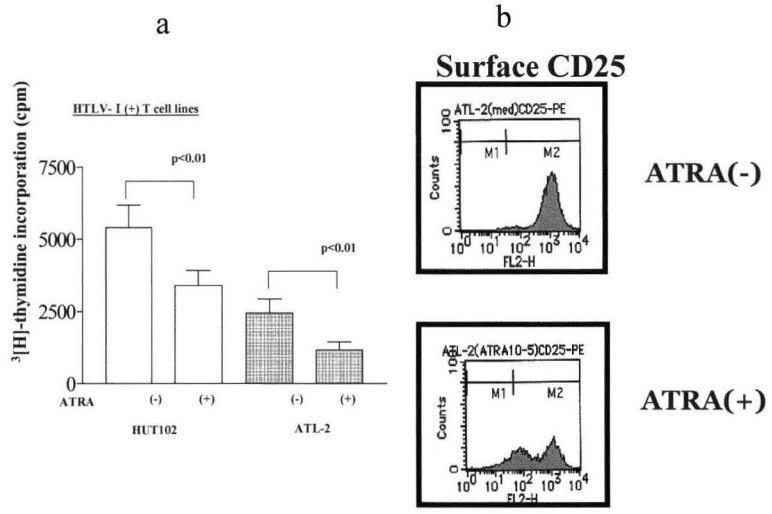

Fig. 1 Effects of ATRA for proliferation and CD25 expression
a : Effects of ATRA on [^{3}H]-thymidine incorporation in ATL-2 cells. Cells were incubated with the indicated concentrations of or ATRA for 48 h and assayed for [^{3}H]-thymidine incorporation. Four different experiments were carried out, and data represent mean$\pm$SD in the figure.
b : Effects of RA on CD25 expression by ATL-2 cells. Cells were incubated the indicated concentrations of ATRA (% positive : medium=92.5, 10^{-5} M=76.8, MFI : medium=112.4, 10^{-5} M=82.5) for 48 h and then assayed for CD25 expression by flow cytometry.

regard to down-regulation of CD25 is not clear. However, this difference may be attributable to : (i) Differences in the expression of retinoic acid receptors (RARs)[32,33] or retinoid X receptors (RXRs),[34,35] these receptors expression may be associated with the sensitivity to RA. (ii) Differences in the expression of cytosolic retinoic acid binding proteins (CRABPs), which binds RA before its transfer to the nucleus and acts as an intracellular antagonist of RA action.[36–39] The extent of CRABP expression would be expected to correlate with RA resistance. And (iii) differences in the expression of anti-oxidant including ATL-derived factor (ADF). Indeed, our study showed that incubation with ATRA for 48 h resulted in inhibition of growth for PBMCs and in induction of apoptosis from some patients with ATL, but not for PBMCs from normal individuals (Figure 3).[40] Thus, there is a possibility that specific target cells of RA may be ATL cells in peripheral blood.

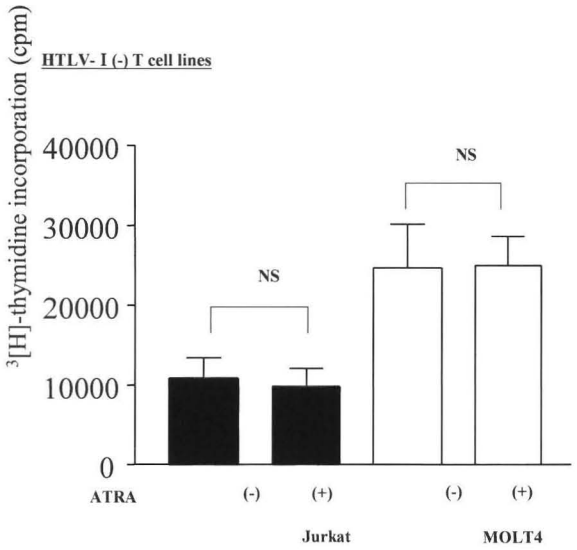

Fig. 2 Effects of ATRA on [^{3}H]-thymidine incorporation in Jurkat and MOLT-4 cells. Cells were incubated with the indicated concentrations of ATRA for 48 h and assayed for [^{3}H]-thymidine incorporation. Four different experiments were carried out, and data represent mean ± SD in the figure.

Inhibition of NF-κB transcription activity

We next investigated NF-κB transcription activity by CAT assay with pCD12-CAT. As shown in Figure 4, spontaneous enhancement of CAT activity for NF-κB was detected. CAT activity determined with percent conversion was decreased after treatment with ATRA (% conversion : 60.8% to 21.0%). These results suggested that growth inhibition and CD25 down-regulation by ATRA occurred via the NF-κB signaling pathway.

Further, we demonstrated typical apoptosis on

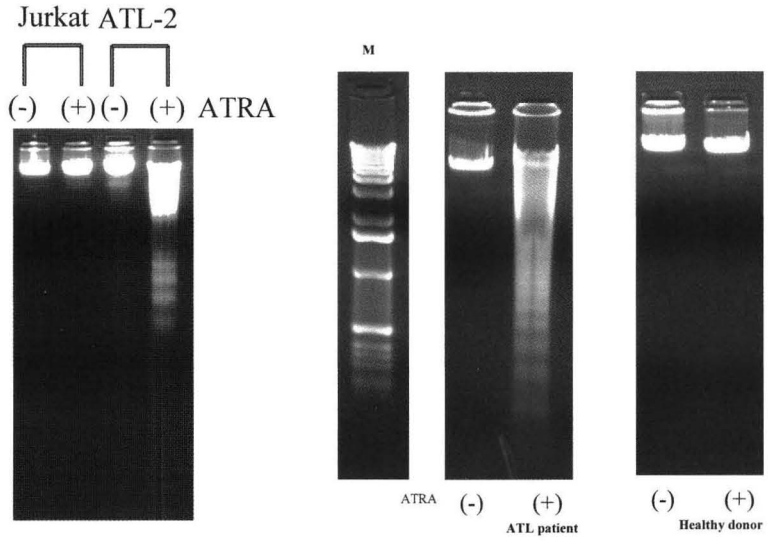

Fig. 3 Induction of apoptosis by ATRA.
Cells were cultured with 10^{-5} M ATRA for 48 hours, DNA was extracted, and electrophoresis with 1.5% agarose gel was performed and visualized by UV irradiation. ATRA induced DNA degradation on ATL-2 cells, but not on Jurkat cells. Induction of apoptosis on PBMCs isolated from a patient with ATL by ATRA. PBMCs were isolated from a ATL patient (acute type No. 1) by Ficoll-Paque density gradient centrifugation. Cells were cultured for 48 hours with 10^{-5} M ATRA, DNA was extracted, and electrophoresis with 1.5% agarose gel was performed and visualized by UV irradiation. ATRA induced apoptosis on PBMCs isolated from acute ATL patients, but not on PBMCs from normal donors.

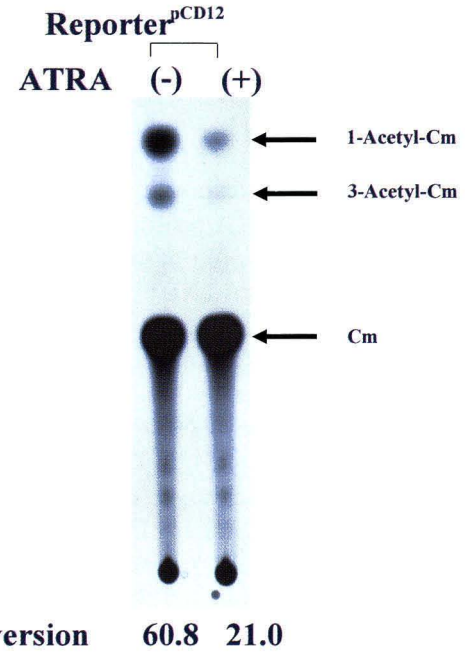

Fig. 4 Relative CAT activity for NF-$\varkappa$B on ATL-2 cells in the presence or absence of ATRA.
pCD12-CAT was transfected into ATL-2 cells by the DEAE-Dextran method. The CAT activity for NF-$\varkappa$B was markedly enhanced in ATL-2 cells without stimulation (% conversion ; 60.8%). When 10^{-5} M ATRA was added to ATL-2 cells for 24 hours, CAT activity was decreased significantly (% conversion : 21.0%).

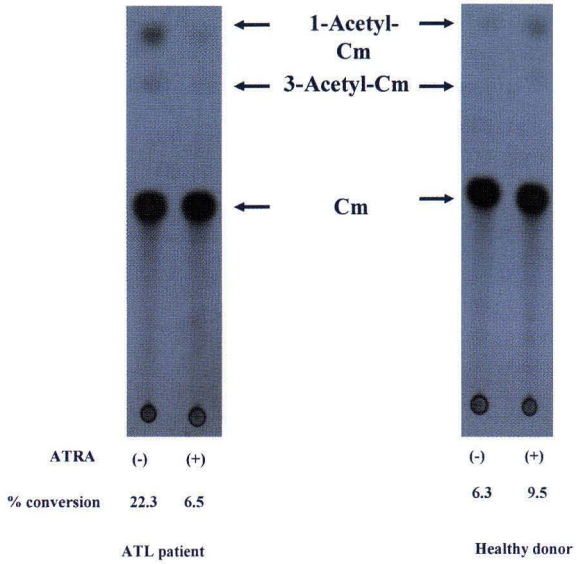

Fig. 5 Inhibition of CAT activity for NF-$\varkappa$B in PBMCs obtained from a fresh acute ATL patient with or without ATRA.
One microgram pCD12-CAT was transfected into PBMCs obtained from a patient with acute ATL and into PBMCs from a normal healthy donor by the DEAE-Dextran method. CAT activity for NF-$\varkappa$B was decreased by treatment with ATRA on PBMCs obtained from the ATL patient (% conversion : 22.3% to 6.5%).

PBMCs obtained from ATL patients after treatment with ATRA for 48 hrs (Figure 3).[41] As shown in Figure 5, CAT-measured NF-$\varkappa$B activity was also significantly decreased on these PBMCs after treatment with ATRA for 24 hrs.

It has been reported that NF-$\varkappa$B is activated by Tax protein, which induces the degradation of I-$\varkappa$Bα, which molecule is known to contribute to constitutive activation of NF-$\varkappa$B in ATL cells for cytokine gene, receptor gene and cell proliferation. We carried out a CAT assay for NF-$\varkappa$B using pCD12-CAT on ATL-2 cells in the presence or absence of ATRA. Enhanced CAT activity determined with percent conversion was detected on ATL-2 cells (% conversion : 60.8%). It has been reported that Tax-mediated increases in NF-$\varkappa$B nuclear translocation result from direct interaction of Tax and MEKK1, leading to enhanced Ikkβ phosphorylation of IkBα.[42,43] Furthermore, Arima et al. reported that Tax is capable of inducing nuclear expression of all four NF-$\varkappa$B species (p50, p55, p75 and p85) in primary ATL cells of acute type patients,[44] and inhibition of apoptosis has been reported to be essential for activation of NF-$\varkappa$B.[45] Our results possible indicate that the enhanced CAT activity for NF-$\varkappa$B may reveal that NF-$\varkappa$B protects against apoptosis. After treatment with ATRA, NF-$\varkappa$B activity decreased significantly (% conversion : 21.0%) on ATL-2 cells. Furthermore, we also transfected the *tax* gene in the expression vector (pCMV-Tax-neo) into the HTLV-I negative T cell line Jurkat, and examined the effects of ATRA on cell growth. Interestingly, ATRA inhibited the growth of these transient transformants, as assessed by ^{3}[H]-thymidine incorporation, but had no effect on the growth of control cells transformed with neomycin-resistance gene alone. Taken together, these results indicate that the difference in the sensitivity to ATRA may be dependent on the expression of Tax. However, Mori et al. have reported that NF-$\varkappa$B constitutively activates in primary ATL cells as well as HTLV-I positive T cell line TL-Om1 independent of Tax protein.[43] In summary, we have shown that ATRA could inhibit growth of the ATL cells and induce their apoptosis with suppressed NF-$\varkappa$B transcriptional activity. These results suggest that the target molecule of ATRA may be Tax or some molecule in the Tax-NF-$\varkappa$B

signaling pathway, and that the existence of Tax would thus enhance the sensitivity to ATRA. Further study will be needed to determine whether ATRA exert its effects directly, or via some intermediary factor. Plans to administer ATRA to ATL patients in a clinical setting were currently undertaken in our laboratory.[46]

Effects of thiol compounds

In ATL, ADF that is homologous to thioredoxin (TRX),[47] have been reported to be not only a CD25 inducer, but also an active reducing molecule for active oxygen species.[47] It was reported that the activity of thioredoxin reductase (TRX-R) from melanoma tissue was inhibited remarkably by 13-*cis* RA.[48] Cellular redox status modulates various aspects of cellular function when oxidative stress occurs. The balance of oxidative/anti-oxidative influences may play an important role in the modulation of cellular function. It has been reported that L-cysteine and L-cystine act as a buffer of the redox potential of the environment in cells or serum.[49,50] To study the effects of exogenous thiol compounds on the sensitivity to retinoid in a HTLV-I (+) T cell line, ATL-2[51] cells were cultured with thiol compounds (10^{-5} M L-cystine, 10^{-4} M GSH and 1 μg/ml TRX), following addition of ATRA or 13-*cis* RA. Significant growth inhibition was seen in ATL-2 cells when 10^{-5} M RA was added. Unexpectedly, similar growth inhibition of ATL-2 cells was shown with each thiol compound added to ATL-2 cells.[52,53] These unexpected results may be explained by differences in uptake time into the cells between RA and thiol compounds. Next, we preincubated ATL-2 cells with each thiol compound (1 μg/ml recombinant ADF, 1 μg/ml TRX, 10^{-5} M L-cystine and 10^{-4} M GSH) for 24 hrs, and 10^{-5} M ATRA or 13-*cis* was added to ATL-2 cells in thiol-depleted medium. Table 1 summarized the effects of preincubation with thiol compounds and the sensitivity to RA determined by reduction rate of [^{3}H]-thymidine incorporation. The reduction rate was decreased significantly by preincubation with the thiol compounds. Especially, preincubation of ATL-2 with L-cyctine or GSH resulted in complete restoration of growth despite the inhibitory effects of RA, this suggested that it helped to increase the redox potential of the intracellular environment. Intracellular L-cystine is converted to L-cysteine, which is an active thiol compound that is utilized for GSH synthesis[49] and depletion of L-cystine results in a reduction of intracellular GSH content. These processes are antagonized by antioxidants such as cysteine and GSH.[50] However, no restoration of growth was obtained in thiol-untreated ATL-2 cells. These reports suggested that L-cystine/GSH and ADF/TRX systems cooperate to support the adjustment of intracellular redox states against several oxidants and, thereby, promote the growth and viability of lymphocytes. Our results suggest that the imbalance of intracellular redox potential in HTLV-I (+) T cell lines may be associated strongly with the sensitivity to RA and exogenous thiol compounds may prepare the intracellular environment to become resistant to RA. In other words, cystine/GSH and ADF/TRX redox systems may act against RA, an antioxidant.

Effects of skin involvement

ATL is characterized by infiltration of various tissues by circulating ATL cells. Especially, skin lesions occur in 50% of ATL patients. In this study, we observed the effects of ATRA on skin involvement in ATL patients. Eight patients with ATL (2 cases acute type, 5 chronic type and

Table 1 The effects of preincubation with thiol compounds and the sensitivity to RA.

	Preincubation				
	Med	rADF	TRX	L-cystine	GSH
Ethanol	5163±183	4013±293	6623±213	6314±397	6482±227
ATRA	2899±28	3214±90	5063±449	6426±66	6396±163
(% reduction)	(43.8%)	(19.9%)	(23.6%)	(−0.018%)	(0.013%)
13-*cis* RA	3650±107	4351±921	5941±906	6414±868	7812±33
(% reduction)	(29.3%)	(−0.08%)	(10.3%)	(−0.015%)	(−20.5%)

The reduction rate (% reduction) was calculated as follows: % reduction=(basal [^{3}H]-thymidine incorporation−[^{3}H]-thymidine incorporation after RA treatment)/(basal [^{3}H]-thymidine incorporation)×100. Values for the calculation were the mean of triplicate culture.

1 smoldering type) were selected for this study. Cutaneous lesions included erythematous plaques, papules, nodules, erythroderma, and tumors. Patients were scheduled to receive oral ATRA 45 mg/m^2 daily. During treatment with ATRA, there was no chemotherapy or glucocorticoid therapy administered. Patients were monitored for safety and anti-tumor effect by regular physical examination and laboratory studies including complete and differential blood count and standard chemistry performed at the baseline and repeated at weeks 1, 2, 3 and 4. Skin biopsy was carried out before and after treatment with ATRA Complete response required all skin eruptions coming macroscopically negative. ATRA was effective for skin involvement in 6 patients. A typical case is shown below ; Case : A 42-year-old Japanese woman was referred to our hospital because of skin eruption with chronic ATL. After detection of proviral DNA in the skin by Southern blot analysis, ATRA (60 mg/day) was administered. The skin biopsy exhibited dense lymphoid infiltrates with atypical cytological features in the dermis. The infiltrate was composed mainly of medium to large cells with irregular nuclei. Neoplastic cells showed mild epidermotropism. There was a clinical and histological improvement after ATRA therapy was given for 4 weeks (Figure 6a). Furthermore, proviral DNA for HTLV-I by Southern blot analysis in skin became to be negative after treatment with ATRA (Figure 6b). These results indicated that ATRA may be a useful agent for skin involvement of ATL. Adverse effects were seen in 6 of 8 patients, these effects were temporally and generally mild (3 cases of headache, 2 cases of dry skin, 1 case of skin pigmentation). This

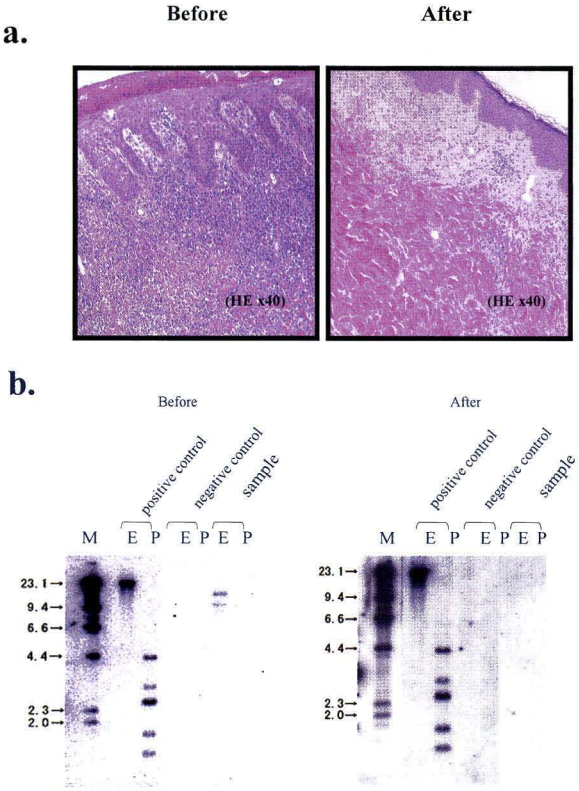

Fig. 6 Effects of ATRA for skin involvement
a) Skin biopsy specimen (hematoxylin and eosin ; original magnification x 40) before and after ATRA treatment, b) Proviral DNA by Southern blot analysis before and after ATRA treatment. Two bands of slightly differing intensity are shown after EcoRI digestion from skin sample before ATRA therapy. After ATRA therapy, there were no integration bands detected. M : molecular marker, E : *EcoRI*, P : *PstI*

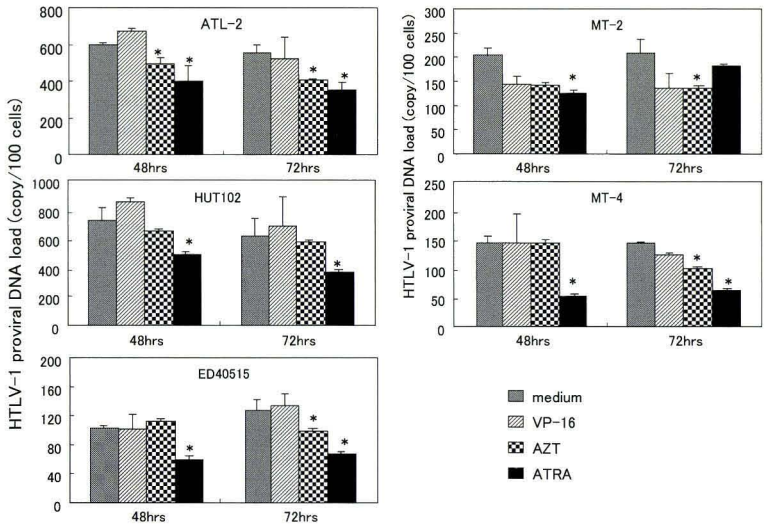

Fig. 7 Inhibition of HTLV-1 proviral DNA load by ATRA
HTLV-1 proviral DNA load was significantly suppressed by AZT in the HTLV-1-positive T-cell lines (ATL-2 and MT-2 at 48 hours, and ATL-2, MT-2, MT-4 and ED40515 at 72 hours). Furthermore, HTLV-1 proviral DNA load was also significantly decreased by ATRA in HTLV-1-positive T-cell lines (all five HTLV-1-positive T-cell lines at 48 hours, and ATL-2, HUT102, MT-4 and ED40515 at 72 hours).

confirms that as it has been reported ATRA only shows toxicities in a few cases. we had 2 cases that did not respond to ATRA, indicative of ATRA resistant cases. Differences between good responders and resistant cases should be investigated, including the mechanism of ATRA action for skin involvement.

Mechanism of ATRA action for ATL cells

At the present time, the mechanism of ATRA' s effect in ATL cells is not clear. In this study, we observed two critical points; 1) whether ATRA suppresses HTLV-1 replication, and 2) whether ATRA decreases RT activity via a direct reaction.

To confirm the anti-retroviral effect of ATRA, detection of HTLV-1 proviral DNA load using real time PCR was carried out in five HTLV-1-positive T-cell lines treated with VP-16, AZT, and ATRA for 48 and 72 hours. As shown in Figure 7, HTLV-1 proviral DNA load was only decreased by VP-16 in MT-2. HTLV-1 proviral DNA load was significantly suppressed by AZT in the HTLV-1-positive T-cell lines (ATL-2 and MT-2 at 48 hours, and ATL-2, MT-2, MT-4 and ED40515 at 72 hours). Furthermore, HTLV-1 proviral DNA load was also significantly decreased by ATRA in HTLV-1-positive T-cell lines (all five HTLV-1-positive T-cell lines at 48 hours, and ATL-2, HUT102, MT-4 and ED40515 at 72 hours). These results suggested that ATRA might act as a RT inhibitor.

Moreover, HTLV-1 tax mRNA load was significantly suppressed by ATRA (HUT102 and MT-2 at 48 hours). However, HTLV-1 tax mRNA load was decreased by ATRA as well as AZT in two HTLV-1-positive T-cell lines. In spite of the significant decrease in HTLV-1 gag or pol mRNA load caused by ATRA or AZT treatment in ATL-2, HTLV-1 tax mRNA load was not reduced by ATRA or AZT treatment in ATL-2. However, both HTLV-1 gag, pol and tax mRNA load decreased in MT-4 treated with AZT, but not ATRA (Figure 8).

As ATRA reduced HTLV-1 proviral DNA load, we observed whether it degrades the RT that participates in the cycle of retroviral replication. HTLV-1-positive T-cell lines (1×10^5/ml : total 20 ml) were cultured with 10^{-5} M ATRA, 64 μM AZT or control reagent. Using the RT detection assay, we measured the RT activity of

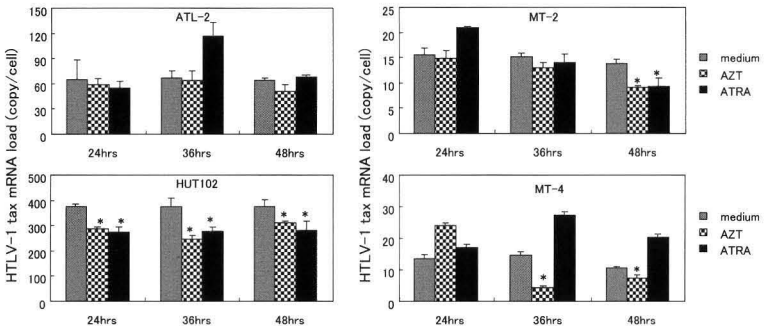

Fig. 8 ATRA decreased HTLV-1 tax mRNA
HTLV-1 tax mRNA load caused by ATRA or AZT treatment in ATL-2, HTLV-1 tax mRNA load was not reduced by ATRA or AZT treatmen.

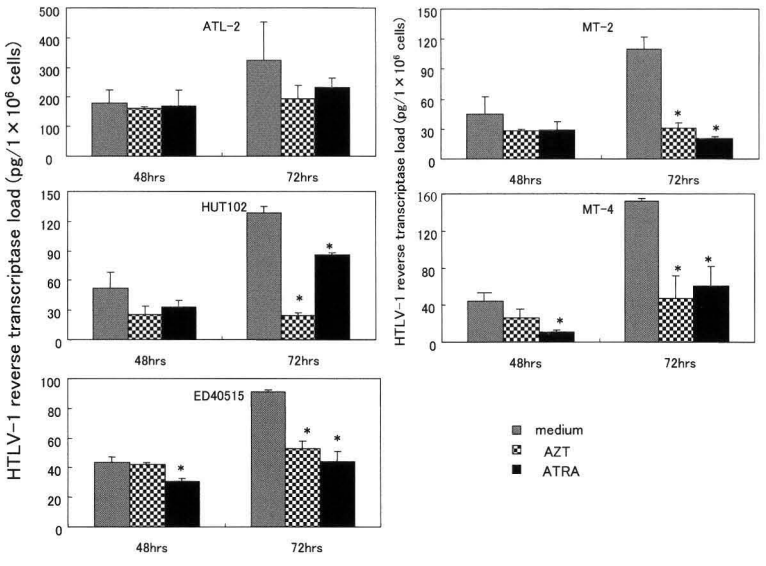

Fig. 9 Effects of ATRA for reverse transcriptase activity
Using the RT detection assay, we measured the RT activity of cell lysates. It was observed that ATRA significantly suppressed the activity in HTLV-1-positive T-cell lines (MT-4 and ED40515 at 48 hours, and HUT102, ED40515, MT-2 and MT-4 at 72 hours)

cell lysates. It was observed that ATRA significantly suppressed the activity in HTLV-1-positive T-cell lines (MT-4 and ED40515 at 48 hours, and HUT102, ED40515, MT-2 and MT-4 at 72 hours) (Figure 9).

In summary, we found that ATRA reduce HTLV-1 proviral DNA at mRNA level and RT activity of HTLV-1. These results suggest that the mechanism of ATRA's action may be dichotomized into inhibition of NF-$\varkappa$B transcriptional activity related to HTLV-1 and inhibition of RT.

Clinical application

We confirmed the clinical effects of ATRA in 20 ATL patients. The median age was 56 years (range, 35-73). In total, 7 men and 13 women were enrolled in the study. Of these, 7 patients presented with the acute type ; 3, lymphoma ; 4, chronic ; and 6, smoldering. The performance status (PS) of the patients ranged between 0 and 2, and 10 patients (50%) had skin involvement and 7 (35%), liver dysfunction. The treatment efficacy was as follows : CR, 0% of the patients ; PR, 40% ; NC, 45% ; and PD, 15% (Table 2). In the 7 acute patients, a PR was achieved in 2 (28.5%) ; NC, 2 (28.5%) ; and a PD, 3 (42.8%). In all the 3 lymphoma-type patients, a PR (100%) was achieved. In the 4 chronic-type patients, a PR was achieved in 1 (25%) and NC was observed in the remaining 3 (75%). Among the 6 smoldering-type patients, a PR was achieved in 2 (33.3%) and NC was observed in 4 (66.6%). As

Table 2 Clinical response and adverse effects of ATRA

UPN	Dose of ATRA (mg/day)	Treatment duration (days)	Response	Duration of response (days)	adverse effect (CTC* grade)	Reasons for discontinuation	Outcome
1	80	30	NC	—	—	NC	Death
2	70	28	PR	31	headache (Grade 2)	worsening	Alive
3	60	56	PR	30	—	worsening	Alive
4	60	14	PR	45	anorexia (Grade 3)	anorexia	Death
5	60	30	NC	—	—	NC	Alive
6	70	26	NC	—	headache (Grade 2)	NC	Alive
7	80	28	NC	—	—	worsening	Death
8	60	20	PD	—	headache (Grade 1)	pneumonia	Death
9	70	14	NC	—	hyperlipidemia (Grade 2)	NC	Alive
10	80	20	NC	—	—	NC	Death
11	80	30	PD	—	headache (Grade 2)	PD	Death
12	70	30	PR	28	—	worsening	Alive
13	80	30	NC	—	liver dysfunction (Grade 2)	NC	Alive
14	80	14	PR	30	liver dysfunction (Grade 2)	liver dysfunction	Alive
15	60	28	PD	—	—	PD	Alive
16	70	30	PR	50	—	transfer to SCT	Alive
17	60	14	NC	—	hyperlipidemia (Grade 1)	worsening	Alive
18	60	28	PR	30	—	transfer to SCT	Alive
19	60	30	PR	29	headache (Grade 1)	transfer to SCT	Alive
20	90	14	NC	—	—	NC	Death

*CTC grade, common terminology criteria for adverse events

shown in Table 2, adverse effects were noted in 10 of the 20 patients (50%). These effects were generally mild (headache in 5 patients; liver dysfunction, 2; hyperlipidemia, 2; and anorexia, 1). No hematological toxicity was observed.

Considering the results described above, we indicated that ATRA has a therapeutic effect on ATL and should be the first choice for treating ATL. However, in fact, the present study showed no CR, which is not consistent with the results obtained in previous *in vitro* studies.[54,55] Interestingly, in the analysis among subtypes, ATL of the lymphoma-type showed a better PR rate than ATL of the acute-type. In conclusion, the causes leading to a favorable response for ATRA treatment remain unknown. However, our clinical trial of ATRA for skin involvement demonstrated that ATRA was effective in the treatment of skin involvement in 6 of 8 patients (74%).[56] Taken together, these results show that ATRA may have potential in the treatment of tumor formation with ATL cells than intravascular ATL cells. The present study showed that some patients are sensitive to ATRA while some are resistant. To elucidate the mechanism of resistance to ATRA, we focused on the intracellular redox potential. The imbalance of the intracellular redox potential in HTLV-I (+) T-cell lines may be strongly associated with the sensitivity to RA, and exogenous thiol compounds may cause the intracellular environment to become resistant to ATRA.[52,53] In one of our recent studies, the mechanism by which ATRA acts on ATL cells were examined. The results showed that the mechanism could be dichotomized into inhibition of the transcriptional activity of NF-$\varkappa$B related to HTLV-I and inhibition of reverse transcriptase.[57] This dichotomy model means multi-target therapy, and indicated that if one pathway is blocked by some factors, the other one will be available. Furthermore, we should recognize the differences between the clinical outcome and experimental results *in vitro*. We examined the differences in several clinical parameters (LDH, AL-P, sIL-2R, and age) between cases of NC and PR. However, no significant difference was observed (data not shown). Other intrinsic factors (i.e., retinoic acid receptor (RAR)-α expression, cellular retinoic acid binding protein (CRABP) expression etc.) need to be investigated carefully. We previously established a myeloid cell line with retinoid resistance. The cells expressed multi drug resistance 1 (MDR-1) mRNA and p-glycoprotein cell surface protein, we assessed whether verapamil and ATRA would induce the differentiation of the cells, however, they did not. An increased expression of cellular retinoic acid-binding protein (CRABP)-II was also detected on the cells compared with that of HL-60. These results suggest that high level of expression of CRABP-II may contribute to be the mechanism of ATRA resistance.[58] Further, serum concentration of ATRA would be an important factor, especially trough level should be measured in each case.

In the present study, the common adverse effects of ATRA were temporal and generally mild (5 patients had headaches, 2 had liver dysfunction, 2 had hyperlipidemia, and 1 had anorexia). Moreover, the adverse effects ranged between CTC grade 1 and 3. As mentioned above, ATRA may be useful in treating some ATL patients and may also be used in combination with other chemical agents. When ATRA used with conventional chemotherapy, we suggested that dose of anti-neoplastic agents could be reduced significantly. Further, the non-myeloablative chemotherapy will be able to reduce the opportunities of severe infection and hemorrhagic disorder in the clinical course.

In conclusion, we firmly believe that treatment with ATRA can provide some benefits to clinicians and ATL patients.

Effects of HIV infection

Finally, we concluded that the mechanism of ATRA's action may be dichotomized into the inhibition of NF-$\varkappa$B's transcriptional activity related to HTLV-1 and inhibition of RT.[57] It was reported that vitamin A supplementation reduced HIV-associated disease and slowed the progression toward AIDS.[59] Maciaszek et al. reported that ATRA repressed HIV-1 long terminal repeat-directed expression in THP-1 monocytes.[60] Furthermore, Hanley et al. reported that a synthetic pan-retinoic acid receptor antagonist, BMS-204 493, activated replication of HIV-1 in a dose-dependent manner.[61] This phenomenon suggested that ATRA-induced transactivation of cellular gene expression is required for the viral replication.[62] On the other hand, it was reported that RA stimulates transcription of HIV in human neuronal cells.[15] As shown in Figure 10, the HIV-1 proviral DNA load in 8E5 cells was significantly reduced by ATRA as well as AZT. Furthermore, ATRA affected viral replication in the three HIV

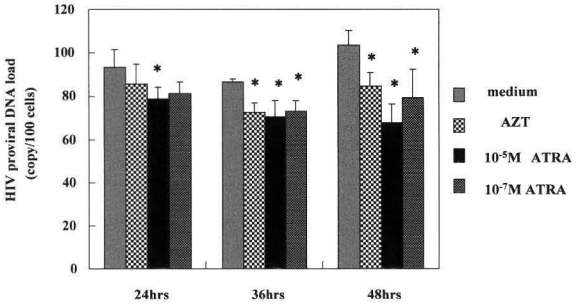

Fig. 10 Inhibition of HIV proviral DNA of 8E5 cells by ATRA
HIV proviral DNA load increased in a time-dependent manner in spontaneous culture. However, ATRA reduced proviral DNA load in both a dose-dependent and time-dependent manner.

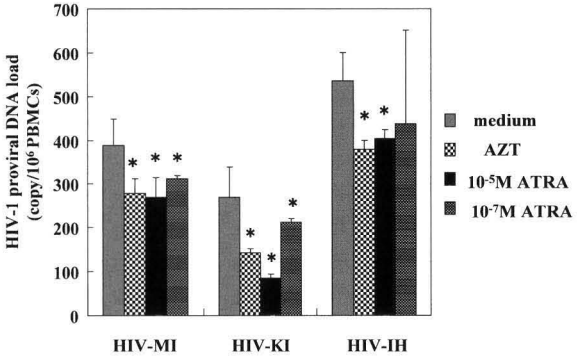

Fig. 11 Inhibition of HIV proviral DNA of fresh HIV patients by ATRA
Three patients with un-treated HIV-1 (HIV-IK, HIV-IM and HIV-KT) were selected in this study. Infection was confirmed using an antibody for HIV-1. The number of HIV-1 RNA copies was 13000 for HIV-IM, 8900 for HIV-IK and 9000 for HIV-KT.

patients. Figure 11 shows HIV proviral DNA load on treatment with AZT, 10^{-5} M ATRA or 10^{-7} M ATRA. Interestingly, ATRA could reduce viral replication not only in the 8E5 cell line but in the primary lymphocytes from HIV patients. Regarding ATRA and HIV infection, there are several interesting reports.[63,64] Briefly, four patients were diagnosed with HIV infection and APL at the same time. The use of HAART was not reported in three of these cases. All three patients with APL and HIV infection treated with ATRA achieved a complete remission.[21] Furthermore, the $CD4^+$ cell count decreased during therapy, but increased once the treatment was completed, and the patient did not suffer any HIV-associated complications.[64] This phenomenon may explain why ATRA affects both APL and HIV infection. Furthermore, a case of APL and ATL associated with HTLV-I infection treated with ATRA was reported.[65] The patient was diagnosed with APL and smoldering ATL simultaneously, and treated with ATRA (60 mg/day p.o.). At day 17 of ATRA treatment, the WBC count was normal with less than 1% APL and ATL cells. Monoclonal integration of HTLV-I was undetectable at that time. Hematological findings showed no abnormality on morphological, phenotypical, cytogenetic and molecular biologic analyses at day 50, when ATRA therapy was discontinued.

Moreover, we examined the effects of ATRA on RT activity. RT activity decreased significantly on treatment with ATRA as well as AZT. The mechanism by which ATRA inhibited HIV replication may be inhibition of RT activity (data not shown). Taken together, ATRA may be a useful therapeutic tool for HIV infection.

Conclusion

We have believed that treatment with ATRA can provide some benefits to clinicians and ATL patients as having based on several evidences. Finally, we hope that ATRA is a useful agent for other HTLV-I-associated disorders, including HAM (HTLV-I-associated myelopathy), HAAP (HTLV-I-associated arthropathy), HAB (HTLV-I-associated bronchopathy) and HAU (HTLV-I-associated uveitis).

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