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Research Article

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Pharmacological Inhibition of In Vitro Infectivity of Human T Lymphotropic Virus Type I

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Abstract

Human T lymphotropic virus type I (HTLV-I) is an exogenous RNA tumor virus etiologically linked to adult T cell leukemia and related diseases. In this paper, we describe that two 2′,3′-dideoxynucleoside analogues, erythro 3′-azido-2′,3′-dideoxythymidine (also called azidothymidine) and 2′,3′-dideoxycytidine can inhibit the infectivity of HTLV-I against helper/inducer T cells in vitro.

Both 2′,3′-dideoxynucleoside analogues inhibited the overgrowth of target T cells, which was a consequence of virally mediated transformation, when they were exposed to the virus and cultured with the compounds. A profound decrease in the expression of HTLV-I *gag*-proteins was also observed. Moreover, we observed that the amount of proviral DNA detected in cellular DNA from the target T cells was substantially reduced when the cells were protected by the compounds against the virus and that at certain concentrations of the compounds the synthesis of viral DNA was completely suppressed. These results may be of value in developing a new pharmacological strategy for preventing the replication and possibly blocking the transmission of HTLV-I and related retroviruses in human beings.

Introduction

Human T lymphotropic virus type I (HTLV-I)[1] is an exogenous RNA tumor virus associated with adult T cell leukemia (ATL) (1–5), a fulminant malignancy endemic in several parts of the world (6, 7), which includes southwestern parts of Japan. In the United States, HTLV-I infection (alone or in combination with other pathogenic retroviruses) appears to be a growing problem, especially among individuals who are addicted to illicit intravenous drugs (8). The West Indies, parts of South America, and several regions of Africa are known to be other endemic areas (3, 4). ATL was first reported as a clinical entity in Japan (9–11). It has recently been recognized that HTLV-I can cause a wide spectrum of diseases, including an immunodeficiency state not necessarily accompanied by leukemia (12). Atypical cases of ATL (13), asymptomatic carriers (14), and neurological abnormalities such as HTLV-I–associated myelopathy (15) have been reported. Also, HTLV-I infection might be associated with tropical spastic paraparesis (16). Although the mechanisms of natural transmission of HTLV-I are not fully understood, seroepidemiological studies suggest horizontal and/or vertical transmission of HTLV-I (4, 6, 7). Moreover, cases of transfusion-associated HTLV-I infections are not uncommon in certain endemic areas (17). These observations have prompted us to attempt to explore strategies for preventing the replication and transmission of HTLV-I (18).

All retroviruses, including HTLV-I, require a viral DNA polymerase called reverse transcriptase (RT) in their natural cycle of replication (19, 20). Mitsuya et al. (21–23) have recently reported that 2′,3′-dideoxynucleoside analogues can inhibit the infectivity and cytopathic effect of another human retrovirus, human T lymphotropic virus type III (HTLV-III) (also called lymphadenopathy-associated virus [LAV] or acquired immunodeficiency syndrome (AIDS)–related virus; recently, the human retrovirus subcommittee of the International Committee on the Taxonomy of Viruses has proposed that AIDS retroviruses by officially designated as human immunodeficiency virus, HIV [24]), which is considered to be the causative agent of AIDS. These in vitro experiments have been done using a dose of virus greatly in excess of the minimal cytolytic dose (21, 22). However, the extrapolation from HTLV-III (a virus that can readily infect target cells by cell-free virions and that has as its hallmark the capacity to destroy helper/inducer T cells) to HTLV-I (a virus that in general requires cell-associated virion transmission for infection and that has as its hallmark the capacity to transform helper/inducer T cells) is not straightforward. In the present study, we investigated the effect of two dideoxynucleoside analogues, erythro 3′-azido-2′,3′-dideoxythymidine (also called azidothymidine [AZT]) and 2′,3′-dideoxycytidine (ddCyd) on the infectivity of HTLV-I by using cloned, normal helper/inducer T cells as target cells. We now report the capacity of AZT and ddCyd to inhibit the infectivity and replication of HTLV-I in vitro.

Methods

Target cells. We have previously described the method for generating normal tetanus-toxoid–specific helper T cell clones (25), and one such normal OKT4$^+$ clone (TM-11) was used as a target cell line for this

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1. *Abbreviations used in this paper:* ATL, adult T cell leukemia; AZT, erythro 3′-azido-2′,3′-dideoxythymidine; ddCyd, 2′,3′-dideoxycytidine; HTLV-I, human T lymphotropic virus type I; HTLV-III, HTLV type III; LAV, lymphadenopathy-associated virus; PBM, peripheral blood mononuclear cells; RT, reverse transcriptase; TCR-β, T cell receptor β-chain; TM-11–H, HTLV-I–infected TM-11 cells.

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study. Briefly, TM-11 was generated from a normal individual whose serum was negative for anti-HTLV-I antibody. TM-11 cells were maintained in 15% (vol/vol) interleukin 2 (IL-2, lectin depleted; Cellular Products, Buffalo, NY)–containing complete medium (RPMI 1640 supplemented with 4 mM L-glutamine; 50 μM 2-mercaptoethanol; 15% undialyzed, heat-inactivated fetal calf serum; and antibiotics). TM-11 cells undergo a proliferative reaction in response to tetanus-toxoid in the presence of irradiated (4,000 rad), autologous peripheral blood mononuclear cells (PBM). This clone displays the following surface phenotypes: $T3^+$, $T4^+$, $T8^-$, HLA-DR^+, and Tac-$antigen^+$.

Nucleosides. AZT was kindly provided by Wellcome Research Laboratory, whereas ddCyd was purchased from Calbiochem-Behring Corp., La Jolla, CA.

Cell culture. Target TM-11 cells were stimulated with antigen plus irradiated, autologous PBM 6 d before the experiment. Growing TM-11 cells (5×10^6) were co-cultured with 10^6 lethally irradiated (12,000 rad), HTLV-I–producing M.J.-tumor cells (26) in 24-well culture plates (Costar, Cambridge, MA) in 2 ml of 15% IL-2–containing complete medium in the presence or absence of various concentrations of the compounds. Cells were continuously exposed to IL-2 and cultured at 37°C in humidified air containing 5% CO_2.

On day 2 of culture, one half of the medium was replaced with fresh medium containing the same concentrations of the compounds. After 3 d of close contact with HTLV-I–producing cells, TM-11 cells were transferred to culture flasks (Costar 3275; Costar) and were resuspended in 40 ml of 15% IL-2–containing complete medium in the presence of the same concentrations of the compounds. The number of viable cells were counted at various time points by the trypan blue exclusion method. On day 9 of culture, higher concentrations of the compounds were reduced: AZT, from 9 and 27 μM to 4.5 and 10 μM, respectively, whereas ddCyd, from 8 μM to 4 μM. Other concentrations were maintained throughout the study. On day 18 when the control virus-unexposed, drug-unexposed cells reached a plateau and stopped replicating, all the cultured populations were analyzed for the expression of viral *gag* proteins (p24 and p19) by an indirect immunofluorescence assay and for the amount of HTLV-I proviral DNA in cellular DNA by Southern blot hybridization (see below). Aliquots of the cells were sometimes frozen, stored in liquid nitrogens, and subsequently thawed.

Detection of HTLV-I gag protein expression. Methanol-acetone–fixed cells were analyzed by an indirect immunofluorescence assay for the presence of HTLV-I *gag* proteins using murine monoclonal antibody specifically against HTLV-I *gag* protein p19 and goat anti-serum against HTLV-I *gag* protein p24 (kind gifts from Dr. M. Robert-Guroff, National Cancer Institute, Bethesda, MD) according to published methods (27, 28).

Southern blot hybridization. Southern blot hybridization was performed as previously described (2). Briefly, high molecular weight DNA was extracted with organic solvents. 40 μg (unless otherwise stated) of DNA were digested for 16 h at 37°C with 60 U of the restriction endonuclease Bam HI (Bethesda Research Laboratories, Gaithersburg, MD). The digests were subjected to electrophoresis overnight at 40 V in 0.8% agarose, transferred to nitrocellulose, and hybridized with a ^{32}P-nick translated HTLV-I probe (lambda MT-2), which detects the whole sequence of HTLV-I (29). Proviral digestion fragments were detected by autoradiography with Kodak AR film using a screen (DuPont Quanta III; DuPont Photo Products, Burbank, CA). Subsequently, the blots were boiled and rehybridized with a T cell receptor β-chain (TCR-β) probe, YTJ2 (30), which contains variable and constant region sequences, and TCR-β digestion fragments were visualized by autoradiography. Relative levels of HTLV-I viral DNA and TCR-β were compared within the dose response by densitometry readings of the exposed film (31).

Antigen-induced T cell activation assays. Washed TM-11 cells (5×10^4) were cultured for 4 d with tetanus-toxoid and 7.5×10^4 irradiated (4,000 rad), autologous PBM in 200 μl of complete medium in round-bottom microtiter culture plates. Cells were exposed to 1 μCi of [^3H]thymidine for the final 8 h and were harvested onto glass fibers, and the incorporated radioactivity was counted.

Results

Growth characteristics of TM-11 cells exposed to HTLV-I and cultured with AZT or ddCyd. To optimize the in vitro infectivity of HTLV-I, we used a proliferating normal helper/inducer T cell clone, TM-11, as a target and HTLV-I–producing tumor cell line (M.J.-tumor) as a source of infectious virions. TM-11 cells had been stimulated with antigen plus autologous PBM 6 d before the experiment and were cultured alone or co-cultured with lethally irradiated M.J.-tumor cells in the presence or absence of various concentrations of AZT and ddCyd. On days 13–16 of culture, the number of TM-11 cells cultured alone reached a plateau and the cells stopped replicating even in the presence of exogenous IL-2, which resulted in a progressive decrease in the number of viable cells, a common phenomenon seen when normal human, antigen-specific helper/inducer T cell clones are cultured (Fig. 1). In contrast, TM-11 cells exposed to HTLV-I and cultured without the dideoxynucleosides continued to proliferate, and this became particularly evident around day 18. This observation suggests that at least some populations in TM-11 cells were infected and transformed by HTLV-I. When TM-11 cells were exposed to HTLV-I and cultured in the presence of AZT or ddCyd, the cells reached a plateau and stopped replicating, a pattern similar to that of the control virus-unexposed, drug-unexposed TM-11 cells (Fig. 1). However, note that at higher concentrations of the drugs the growth of TM-11 cells appeared to be suppressed and some concentrations were reduced on day 9 and after being in culture (see Methods).

Inhibition of HTLV-I gag protein expression by AZT and ddCyd. On day 18 of culture when the growth curves showed

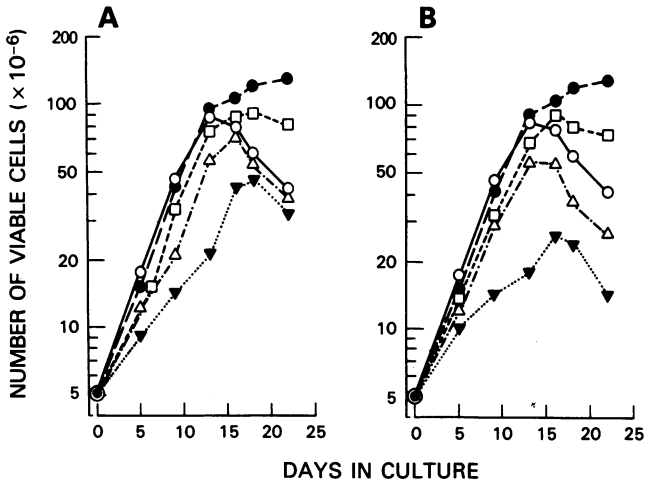

Figure 1. Growth curve of TM-11 cells cultured with AZT or ddCyd when exposed to HTLV-I. Target TM-11 cells had been stimulated with antigen and irradiated, autologous PBM 6 d before the experiment. Cells were cultured alone (○); exposed to HTLV-I but not to the compounds (●); exposed to the virus and 3 μM (□), 9 μM (△), and 27 μM (▼) AZT (*A*); or exposed to the virus and 0.5 μM (□), 2 μM (△), and 8 μM (▼) ddCyd (*B*). On day 9 of culture, higher concentrations of the compounds were reduced; AZT was reduced from 9 μM and 27 μM to 4.5 μM and 10 μM, respectively, whereas ddCyd was reduced from 8 μM to 4 μM. Note that the cells exposed only to HTLV-I continued to grow, but the cells exposed to HTLV-I plus the compounds (≥ 9 μM AZT and ≥ 2 μM ddCyd) apparently stopped the replication on days 13–16 of culture.

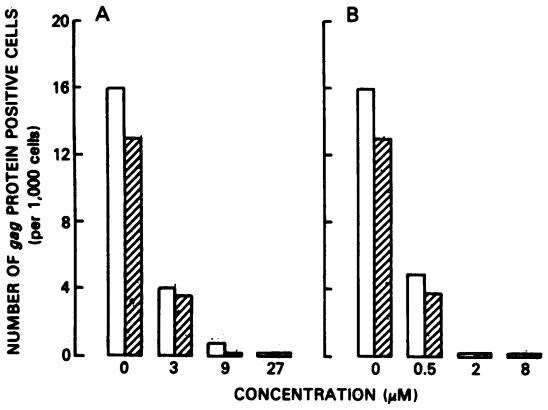

Figure 2. Inhibition of HTLV-I *gag* protein expression by AZT and ddCyd. Normal helper/inducer T cells (TM-11) were exposed to HTLV-I in the presence or absence of various concentrations of AZT (*A*) or ddCyd (*B*). On day 18 of culture, cells were harvested, fixed with methanol-aceton, and analyzed for the expression of HTLV-I *gag* proteins by indirect immunofluorescence assay. More than 1,000 cells were analyzed in each sample, and data are expressed as the numbers of p19 (□) or p24 (■) *gag* protein–positive cells. TM-11 cells cultured alone (not exposed to HTLV-I) were negative for the expression of *gag* proteins.

substantial differences in the number of TM-11 cells, we harvested the cells and assessed them for the expression of HTLV-I *gag* proteins (Fig. 2). TM-11 cells cultured alone were negative for the expression of *gag* proteins. When TM-11 cells were exposed to HTLV-I and cultured in the absence of the compounds, 16 and 13 cells per 1,000 TM-11 cells were found to express viral *gag* proteins p19 and p24, respectively. In the presence of 3 μM AZT or 0.5 μM ddCyd, a substantial decrease of the *gag* protein expression was observed. When the cells were exposed to HTLV-I and cultured at higher concentrations of the compounds, virtually no *gag* protein expression could be detected (Fig. 2). Numbers of the *gag* protein–positive cells in the populations exposed to the virus in the absence of dideoxynucleosides appeared not to be as high as could be expected from the differences in the growth curves of HTLV-I–exposed and –unexposed TM-11 cell populations. As has been observed in other settings (4, 25, 32), it would appear that there is some restriction of viral *gag* protein expression in these cells.

AZT and ddCyd inhibit HTLV-I infection detected as proviral DNA. Since certain cells may not express the viral components even if they bear integrated HTLV-I proviruses (4, 25, 32), we examined proviral DNA in cellular DNA using Southern blot analysis. On day 18 of culture, high molecular weight DNA was extracted from each cell population and was assessed for the presence of HTLV-I proviral DNA. DNA from each cell population was digested with Bam HI (which cuts three times within the HTLV-I–M.J. provirus, yielding a 4.0-kilobase (kb), *gag-pol*–containing internal fragment, a 1-kb *env*-containing internal fragment, and 3′ and 5′ junctional fragments) and hybridized with a radiolabeled HTLV-I–specific probe. No proviral DNA was found in DNA from normal TM-11 cells that were cultured alone (Fig. 3, *I-A*, lane *a*). When cells were cultured with M.J.-tumor cells, polyclonally integrated HTLV-I proviruses were detected as a diffuse, indistinct smear of junctional fragments and a 4-kb internal fragment (Fig. 3, *I-A*, lane *b*). However, in the presence of 3 μM AZT, a substantial decrease of the amount of proviral DNA was observed in the Southern blot analysis (Fig. 3, *I-A*, lane *c*). Densitometry readings of the exposed film showed an 87% decrease in the amount of proviral DNA at 3 μM AZT, with very little apparent toxicity to the cells due to AZT per se (Fig. 1). At higher concentrations of AZT (9 and 27 μM; after day 9, culture reduced to 4.5 and 9 μM), no proviral DNA was detected (Fig. 3, *I-A*, lanes *d* and *e*). Densitometry readings of the exposed film confirmed a complete (100%)

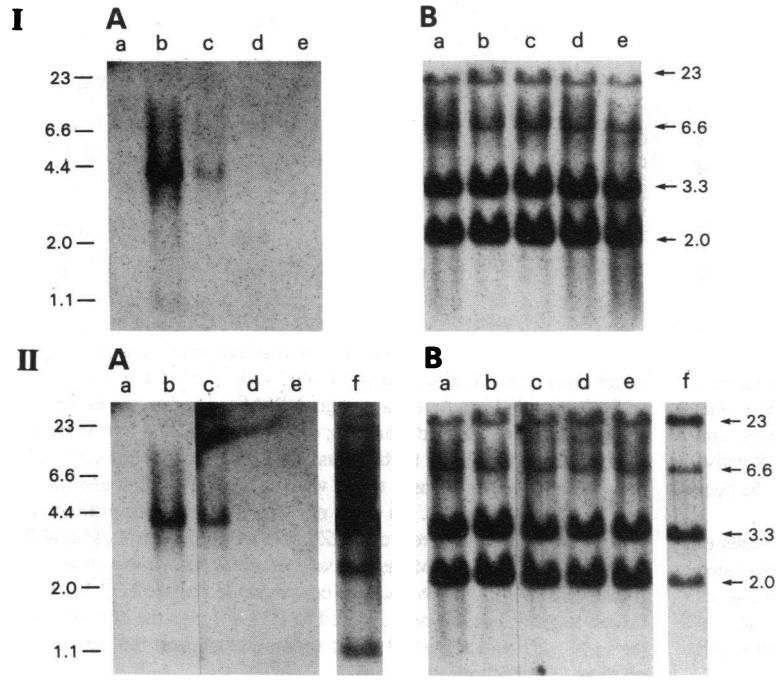

Figure 3. The effect of AZT and ddCyd on proviral DNA synthesis in susceptible T cells exposed to HTLV-I. On day 18 of culture, high molecular DNA was extracted from TM-11 cells. 40 μg of each DNA preparation was digested with Bam HI and was analyzed by Southern blot hybridization using a radiolabeled HTLV-I probe (*I-A* and *II-A*) or TCR-β probe (*I-B* and *II-B*). The cells had been cultured alone (lane *a* in *I-A* and *II-A*); exposed to HTLV-I in the absence of the compounds (lane *b* in *I-A* and *II-A*); exposed to HTLV-I in the presence of 3 μM (*I-A*, lane *c*), 9 μM (*I-A*, lane *d*), and 27 μM (*I-A*, lane *e*) AZT; or exposed to HTLV-I in the presence of 0.5 μM (*II-A*, lane *c*), 2 μM (*II-A*, lane *d*), and 8 μM (*II-A*, lane *e*) ddCyd. Note that when TM-11 cells were cultured alone, no proviral DNA was detected, whereas when the target cells were exposed to HTLV-I, polyclonally integrated HTLV-I proviral DNA was detected as a diffuse, indistinct smear (junctional fragments) and a 4-kb internal fragment. Substantial inhibition of proviral DNA synthesis in the presence of either of the dideoxynucleosides was observed. The filters were then rehybridized with a TCR-β probe (*I-B* and *II-B*), which confirms that the amount of loaded DNA in each lane was essentially equal. DNA from MJ-tumor cells (20 μg) served as a control to show multiple integration of HTLV-I (*II-A*, lane *f*).

suppression of the HTLV-I viral DNA synthesis. Note that although the growth of TM-11 cells were suppressed at higher concentrations of AZT by day 9 of culture, the numbers of viable TM-11 cells became roughly comparable by day ≥ 18 in culture (Fig. 1). The same blot was rehybridized with a radiolabeled TCR-β probe as a control indicator of the amounts of DNA used in the Southern blot experiments (Fig. 3, *I-B*). The TCR-β gene of TM-11 cells appeared in the germline configuration in this particular case using Bam HI digestion. The density of the hybridized bands of TCR-β DNA was virtually comparable in each lane, confirming that approximately the same amount of DNA was loaded in each lane.

We then assessed the anti-viral effect of ddCyd in the same system. As shown in Fig. 3, *II-A*, lane *c*, when TM-11 cells were exposed to HTLV-I and cultured in the presence of 0.5 μM ddCyd, a moderate decrease of the amounts of HTLV-I provirus was observed, and densitometry reading of the film showed a 50% decrease in the amount of HTLV-I proviral DNA. At the same time, there was little or no drug-induced toxicity (Fig. 1). In the presence of 2 and 8 μM ddCyd, no proviral DNA could be detected (Fig. 3, *II-A*, lanes *d* and *e*), and densitometry confirmed a complete inhibition of the HTLV-I viral DNA synthesis. Consider that at these concentrations ~ 40–60% decrease in numbers of viable TM-11 cells was noted on day 18 (Fig. 1). When the DNA from MJ-tumor cells (a source of infectious virions) was hybridized with the HTLV-I probe, after Bam HI digestion, a multiple polyclonal integration of HTLV-I proviruses was observed (Fig. 3, *II-A*, lane *f*). This pattern is commonly seen in cultured HTLV-I-producing cells. The same blot was then rehybridized with the TCR-β probe (Fig. 3, *II-B*). Results indicate that the amounts of cellular DNA loaded were essentially equal (except lane *f* in which half the amount of DNA was loaded). The presence of proviral DNA reflects actual infection by the virus; therefore, these data suggest that AZT and ddCyd blocked in vitro HTLV-I infection of normal helper/inducer T cells.

On day 22 of culture, each TM-11 cell population was extensively washed to remove the dideoxynucleosides and continuously cultured in the absence of the compounds. The cells exposed to HTLV-I and cultured without the compounds as well as the cells exposed to HTLV-I and cultured with 3 μM AZT or 0.5 μM ddCyd could be maintained for ≥ 6 mo without further antigenic stimulation. However, TM-11 cells exposed to HTLV-I and cultured with 9 and 27 μM AZT or 2 and 8 μM ddCyd failed to continuously replicate on days 13–16 and then died out by day 40 of culture even in the presence of exogenous IL-2 as did the control HTLV-I-unexposed cells (data not shown).

Helper/inducer T cells protected by ddCyd remain immunologically competent. When helper/inducer as well as killer/suppressor T cells are infected and transformed by HTLV-I, they often lose their dependence on IL-2, replicate spontaneously, and tend to lose their immunoreactivities (25, 32–35, Matsushita, S., H. Mitsuya, M. S. Reitz, J. B. Trepel, R. F. Jarrett, K. Takatsuki, and S. Broder, manuscript in preparation). We then asked if the T cells protected by dideoxynucleosides against the infectivity of HTLV-I retained their dependence on IL-2 and normal immune reactivities. The TM-11 populations, which had been exposed to HTLV-I, cultured in the absence of ddCyd, and then harvested on day 20 of culture showed both spontaneous and tetanus-toxoid–induced replication in the presence of irradiated, autologous PBM as a source of accessory cells (Table I, Exp. 1). In contrast, TM-11 cells, which had been exposed to HTLV-I, but had been protected by ddCyd and had borne no detectable HTLV-I provirus as described above, proliferated only in response to tetanus-toxoid and did not show a spontaneous replication. The magnitude of the antigen-induced response of the TM-11 cells "protected" by ddCyd was largely comparable to that of HTLV-I–unexposed and ddCyd-unexposed TM-11 populations. We continued the culture of TM-11 cells infected with HTLV-I (TM-11–H) for an additional 8 wk and asked if TM-11–H retained the capacity to respond to antigen for a prolonged period of time after HTLV-I infection (Table I, Exp. 2). Data indicate that TM-11–H exhibited only spontaneous replication (in the absence of IL-2) and showed no capacity to proliferate in response to antigen, as we and other investigators have reported in previous work (25, 32–35). These results suggest that helper/inducer T cells, exposed to HTLV-I that would result in infection, transformation, and loss of antigen-specific immune reactivity, but protected by ddCyd, retain the normal dependence on IL-2 and the capacity to respond to antigen in vitro.

Discussion

Because a rapid and efficient system for in vitro transmission of HTLV-I has not yet become available, it has been difficult to

Table I. Tetanus-Toxoid–induced Proliferation of Helper/Inducer TM-11 Cells Exposed to HTLV-I and Protected by ddCyd

	Exposure to HTLV-I	Protection by ddCyd	Days in culture after exposure	Tetanus-toxoid (limiting flocculation)		
				0	0.5	2
				U/ml	*U/ml*	*U/ml*
Exp. 1	−	−	20	558±94	7,784±884	8,220±1,493
	+	−	20	10,551±1,879	16,429±1,628	18,113±1,031
	+	+	20	535±223	6,254±322	6,467±999
Exp. 2	−	−	25	626±113	19,857±2,906	19,670±684
	+	−	79	111,080±8,391	117,784±11,386	102,166±13,411

TM-11 cells (5×10^4), which had been exposed to HTLV-I and cultured in the presence and absence of 2 μM ddCyd, were cultured in the presence or absence of tetanus-toxoid and irradiated, autologous PBM for 4 d. HTLV-I–unexposed, drug-unexposed TM-11 cells served as a control population. Cells were exposed to [^{3}H]thymidine for the final 8 h and were harvested onto glass fibers, and the incorporated radioactivity was counted. Data are expressed as the arithmetic mean counts per minute±standard deviation of triplicate determinations.

assess the effect of putative anti-viral drugs against HTLV-I. In this study, we attempted to establish a system to analyze the effect of possible anti-viral agents on the infectivity of HTLV-I in a relatively short time period by using an antigen-specific helper/inducer T cell clone as a target population. Since certain cells may not express the viral components even if they bear integrated HTLV-I proviruses (25, 32), we examined total proviral DNA in a given amount of cellular DNA by Southern blot hybridization. The data reported here suggest that two 2',3'-dideoxynucleoside analogues, AZT and ddCyd, have the potential to function as an anti-viral agent against the infectivity of HTLV-I in vitro. Although at higher concentrations of AZT and ddCyd, moderate to substantial cytotoxicity was observed, decreasing the dosage lessened the toxicity and the TM-11 cells could continuously grow with virtually the same growth pattern as the control virus-unexposed and drug-unexposed cells. We observed an almost complete inhibition of proviral DNA synthesis in the target cells exposed to HTLV-I in the presence of ≥ 9 μM of AZT or ≥ 2 μM of ddCyd as assessed by Southern blot hybridization technique. At these concentrations both compounds also clearly blocked viral replication by using the expression of HTLV-I *gag* protein in the cells as an index of HTLV-I replication. Moreover, the compounds appeared to block the virus-induced transformation in the target helper/inducer T cells without affecting their immunoreactivities. These data suggest that there is a range of the drugs that can provide a protection against HTLV-I without unacceptable toxicity at least in vitro.

The capacity of 2',3'-dideoxynucleosides to potentially inhibit HTLV-I replication and HTLV-I-mediated transformation is important from several points of view. Ongoing replication of human pathogenic retroviruses could be important in certain human diseases (21–23, 36). Moreover, the capacity of these drugs to inhibit HTLV-I-mediated transformation in vitro could conceivably shed light on how this virus acts as an oncogenic agent in vivo. The transformation event might be linked to HTLV-I infection per se. On the other hand, it is conceivable that a process of chromosome-mediated transfer of (unregulated) genes after fusion events between donor and recipient cells brings about the transformed state. If the former mechanism is operative, transformation should be sensitive to chain-terminating nucleoside inhibitors of RT. If the latter mechanism is operative, this would not be the case. Our data are compatible with the idea that HTLV-I infection, rather than chromosome-mediated transfer of genes alone, is responsible for the transforming properties of the virus.

It should be noted that AZT and ddCyd are not new chemicals, and pioneering studies on these compounds were initiated in the 1960s and 1970s, before human retroviruses were proven to exist (37–42). At least one mechanism by which such agents function is to compete with the normal nucleotide counterpart for incorporation into retroviral DNA. These agents must undergo anabolic phosphorylation by host cell kinases before they can mediate an anti-retroviral effect. When the 3'-hydroxyl group of the deoxynucleosides is substituted by hydrogen or other groups, it is not possible for the corresponding nucleotide to form the 5' → 3' phosphodiester linkages necessary for DNA elongation in the replication of retrovirus. There are data that (*i*) AZT and ddCyd are converted to 5'-triphosphates by cellular enzymes (43, 44), (*ii*) the triphosphate products are utilized by HTLV-III/LAV DNA polymerase (or RT) and inhibit DNA synthesis mediated by purified HTLV-III/LAV RT (45), and (*iii*) cellular DNA polymerase alpha is rather resistant to these dideoxynucleosides as a triphosphate form (42). Furthermore, (*iv*) in previous work, the data suggest that at concentrations that are achievable in human cells, dideoxynucleoside-5'-triphosphates can serve as substrate for the HTLV-III RT to elongate a DNA chain by one residue, after which the chain is terminated (45). Certain dideoxynucleosides can thus act as DNA chain-terminators in human retroviral DNA synthesis, although this need not be the only mechanism for their anti-retroviral activity.

2',3'-Dideoxynucleosides can potentially have an effect against virtually any retrovirus, provided that host target cells can supply the appropriate level of anabolic phosphorylation. Recently we have learned that 2',3'-dideoxynucleoside analogues can profoundly suppress the replication of animal lentiviruses such as caprine arthritis–encephalitis virus and equine infectious anemia virus, as well as the focus formation induced by a transforming murine type C retrovirus, Kirsten murine sarcoma virus, in a system that requires only a single-round viral DNA formation and integration (46).

Seroepidemiological studies on HTLV-I have suggested that sexual transmission (originally from husband to wife) and mother-to-child transmission represent common modes of spreading of HTLV-I (4, 6, 7). Transfusion-associated transmission is another mode of viral transmission (17). It is possible that AZT and ddCyd or other related agents could be used in certain clinical setings to block the infectivity of HTLV-I, and thereby to suppress the transmissibility of the virus. It is also possible that anti-viral drugs may have a role in prophylaxis or treatment of HTLV-I-associated diseases if ongoing replication of HTLV-I has clinical relevance as seen in certain patients who appear to be in remission or preleukemic stage (36). Taken together, our observations may be of value in developing a new pharmacological strategy for preventing the replication and blocking the transmission of HTLV-I and related pathogenic retroviruses in human beings (47–49).

Acknowledgments

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