Factors Secreted by Human T Lymphotropic Virus Type I (HTLV-I)-infected Cells Can Enhance or Inhibit Replication of HIV-1 in HTLV-I-uninfected Cells: Implications for In Vivo Coinfection with HTLV-I and HIV-1

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Summary

It remains controversial whether human T lymphotropic virus type I (HTLV-I) coinfection leads to more rapid progression of human immunodeficiency virus (HIV) disease in dually infected individuals. To investigate whether HTLV-I infection of certain cells can modulate HIV-1 infection of surrounding cells, primary CD4$^+$ T cells were treated with cell-free supernatants from HTLV-I-infected MT-2 cell cultures. The primary CD4$^+$ T cells became resistant to macrophage (M)-tropic HIV-1 but highly susceptible to T cell (T)-tropic HIV-1. The CC chemokines RANTES (regulated on activation, normal T cell expressed and secreted), macrophage inflammatory protein (MIP)-1α, and MIP-1β in the MT-2 cell supernatants were identified as the major suppressive factors for M-tropic HIV-1 as well as the enhancers of T-tropic HIV-1 infection, whereas soluble Tax protein increased susceptibility to both M- and T-tropic HIV-1. The effect of Tax or CC chemokines on T-tropic HIV-1 was mediated, at least in part, by increasing HIV Env-mediated fusogenicity. Our data suggest that the net effect of HTLV-I coinfection in HIV-infected individuals favors the transition from M- to T-tropic HIV phenotype, which is generally indicative of progressive HIV disease.

Key words: HIV • HTLV-I • Tax • chemokines • chemokine receptors

During the natural course of human immunodeficiency virus (HIV) infection, a transition of HIV phenotypes has been observed (1–3). During primary infection and the clinically latent period, most HIV isolates are macrophage (M)-tropic (4), whereas in the advanced stage of HIV disease more cytopathic, T cell (T)-tropic viruses predominate (1–3). However, the host or environmental factors affecting such a transition and the reason why HIV disease progresses more rapidly in certain individuals remain unclear.

The effects of coinfection with other pathogens on the pathogenesis of HIV-1 disease have been extensively studied over the past decade. For example, a number of viral transactivators have been shown to upregulate expression from the HIV-1 LTR (5–8), and more recently, a human cytomegalovirus (HCMV)\(^1\)-encoded chemokine receptor was found to serve as an HIV-1 entry cofactor (9). Although these in vitro studies provide important mechanistic information, the effects mediated by those pathogens required coinfection of the pathogens and HIV-1 in the same cell, a phenomenon that is considered to occur rarely in vivo.

Several laboratory and epidemiologic studies have suggested that human T lymphotropic virus type I (HTLV-I) infection exacerbates the cytopathic effects of HIV infection and accelerates the clinical progression of HIV disease in coinfected individuals (10–16); however, other studies have not confirmed these observations (17). To determine

\(^1\)Abbreviations used in this paper: GST, glutathione S-transferase; HCMV, human cytomegalovirus; HTLV-I, human T lymphotropic virus type I; MIP, macrophage inflammatory protein; MOI, multiplicity of infection; RANTES, regulated on activation, normal T cell expressed and secreted; RT, reverse transcriptase; rVV, recombinant vaccinia virus.

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the potential mechanisms whereby HTLV-I infection might modulate HIV-1 infection in dually infected individuals, we used in vitro models consisting of primary CD4+ T cells either cocultured with HTLV-I–transformed MT-2 cells in a transwell system or incubated in the presence of cell-free supernatants from MT-2 cell cultures. We demonstrate that crude supernatants from MT-2 cell cultures inhibit replication of M-tropic HIV-1, but enhance that of T- or dual-tropic HIV-1. In addition, the CC chemokines RANTES (regulated on activation normal T cell expressed and secreted), macrophage inflammatory protein (MIP)-1α, and MIP-1β in the supernatants of the MT-2 cell cultures were identified as the major suppressive factors for M-tropic HIV-1 as well as the positively regulating factors for T-tropic HIV-1. Furthermore, soluble Tax protein was shown to be a positively regulating factor for both HIV-1 phenotypes. The effect of Tax or CC chemokines is mediated, at least in part, by enhancing HIV-1 Env-mediated fusogenic activity. This study suggests that HTLV-I coinfection in HIV-infected individuals may facilitate transition from an M- to a T-tropic HIV phenotype, which is generally indicative of progression to an advanced stage of HIV disease.

Materials and Methods

CD4+ T cells. HTLV-I–transformed MT-2 (18) and HUT-102 (19) cells were provided by G. Franchini (National Cancer Institute, NIH, Bethesda, MD) and grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; Gibco BRL, Gaithersburg, MD). JPK9 cells and JPK/X cells were Jurkat cells expressing the wild-type or mutant form, respectively, of HTLV-I Tax under the control of the metallothionein promoter (20, 21). Expression of Tax in these cells was induced by treatment of cells with 10 μM CdCl2 for 2 d.

PBMCs were isolated from healthy volunteers seronegative for both HIV and HTLV-I, as previously described (22), and CD4+ T cells were negatively selected by column exclusion (CD4+ subset enrichment columns; R & D Systems, Minneapolis, MN). Purify of CD4+ T cells was 95% or more, determined by flow cytometric analysis (data not shown).

Propagation of M-T2-conditioned Medium. Crude supernatants from MT-2 cell cultures were clarified by low-speed centrifugation (3,000 rpm, 30 min) and filtered through 0.2-μm filters to remove cells. The medium (5 ml/ aliquot) was incubated with either control rabbit serum (20 μl), anti-Tax antiserum (20 μl [reference 23]), or a mixture of monoclonal antibodies to RANTES, MIP-1α, and MIP-1β (50 μg/ml each; R & D Systems) at 4°C for 2 h, followed by protein A/G sepharose (UltraLink Immobilized Protein A/G; Pierce, Rockford, IL). Immune complexes bound on the sepharose were removed by extensive washing. The presence of soluble Tax protein in the medium was demonstrated by immunoprecipitation using anti-Tax serum and protein A/G sepharose, and the concentrations of CC chemokines in the medium were determined by ELISAs using commercially available reagents (Promega, Madison, WI). Where indicated, the supernatants were ultracentrifuged at 20,000 rpm for 1 h to pellet HTLV-I virions. Crude supernatants from HUT-102 or A3.01 cells were also propagated in a similar manner.

Purification of Recombinant Tax Protein. Expression in E. coli DH5a Strain and HTLV-I Particles from MT-2 Cells. Tax protein was expressed in E. coli DH5a strain transformed with pGST-Tax (provided by K. T. Jeang, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD), and purified as previously described (24). As a control, glutathione S-transferase (GST) protein was also expressed and purified in the same manner. Both preparations were diazoylated and filtered, and protein concentrations were measured by colorimetric assays (Bio-Rad Laboratories, Hercules, CA). Levels of contaminated endotoxin in the preparations were <10 ng/ml protein (Limulus Amoeocyte Lysate Test; BioWhittaker, Inc., Walkersville, MD). Purity and identity of the proteins were determined by SDS-PAGE followed by Coomassie blue staining and Western blotting using anti-Tax antisera (21,000, respectively. In some experiments, the GST-Tax preparation was treated with anti-Tax serum followed by protein A/G sepharose to specifically remove GST-Tax fusion protein. Nuclear extracts were prepared from CD4+ T cells treated with either GST or GST-Tax as previously described (24), and were analyzed by Western blotting to monitor cellular uptake of the protein.

HTLV-I particles were purified from MT-2 cell culture supernatants as previously described (25).

Virus Strains and Infection. The following virus stocks were propagated by transfecting 293T cells with plasmids encoding the respective molecular clones: NL4-3 (T-tropic [reference 26]; 89.6 (dual-tropic [reference 27]); and ADA8 (M-tropic [reference 28]). Approximately 2 × 10⁶ CD4+ T cells were either pretreated with 50% MT-2-conditioned medium or control medium (A3.01-conditioned medium) or cocultured with MT-2 cells in a transwell system that separates the two cell populations by 0.2-μm pore membrane for 3 d, and then infected with the above molecular clone stock at a multiplicity of infection (MOI) of ~0.05. Approximately half of each infected cell culture supernatant was replaced with the same medium every 4 d, and reverse transcriptase (RT) activity in the supernatants was measured as previously described (29).

Single-round Virus Replication Assays. NL4-3-Luc-R-E− virus stocks pseudotyped by various Envs were generated by transfecting 293T cells with pNL4-3-Luc-R-E− and plasmids expressing Env from either HXB2 (T-tropic), 89.6 (dual-tropic [reference 30]), ADA8 (M-tropic [reference 30]) or amphotropic murine leukemia virus (AMV), as previously described (31). Approximately 10⁵ primary CD4+ T cells were infected with the above luciferase reporter virus (5 × 10⁵ cpm RT activity), and luciferase activity of the cell lysates was measured 4 d after infection using commercially available reagents (Promega, Madison, WI).

Fusion Assays. Recombinant vaccinia virus (rVV)-based cell fusion assays were performed as previously described (32, 33). In brief, primary CD4+ T cells (fusion targets) were infected with vTF7-3 (expressing T7 RNA polymerase) at an MOI of 10; as fusion effectors, BSC-1 cells or CD4+ T cells were infected with vCB21R (encoding the laZ gene driven by the T7 promoter) as well as rVV expressing the mutant HIV Env (vCB16), wild-type IIIB (T-tropic) Env (vCB41), or Ba-L (M-tropic) Env (vCB43), each at an MOI of 10. Cells were incubated at 31°C overnight, and both fusion targets and fusion effectors were mixed per well in 96-well flat-bottomed microwell plates with 40 μg/ml of cytosine arabinoside. After 4 h at 37°C, β-galactosidase activity in the cell lysates was assayed by measuring absorbance at 570 nm using a microtiter absorbance reader (Molecular Dynamics, Sunnyvale, CA).

Results and Discussion

HTLV-I–transformed MT-2 Cells Produce Soluble Factor(s) that Inhibits Infection of Primary CD4+ T Cells with M-tropic HIV-1 and that Enhance Replication of T-tropic HIV-1. The ability of HTLV-I to modulate HIV-1 infection with-
out coinfection of the same cell was initially evaluated by coculturing primary CD4⁺ T cells with MT-2 cells in a transwell system. Primary CD4⁺ T cells isolated from HIV/HTLV seronegative individuals either were untreated or were cocultured with MT-2 cells in a transwell (0.2-μm pore membrane) culture for 3 d, and infected with either HIV-1ADA (A) or HIV-1NL4-3 (B). Cell-free supernatants were collected on days 4, 8, and 12 after infection and assayed for RT activity. Experiments were repeated twice with similar results.

The above results suggested that MT-2-conditioned medium contains soluble factor(s) that suppress M-tropic but enhance T-tropic HIV-1 infection; however, neither the positive nor the negative effects persisted beyond a few days after exposure to the supernatants. To provide continuous exposure of CD4⁺ T cells to the putative factors, crude cell-free supernatants were prepared from MT-2 cell cultures. Primary CD4⁺ T cells were pretreated for 3 d and then continuously exposed to a 1:1 dilution of the supernatants after infection with T-tropic HIV-1NL4-3, dual-tropic HIV-1BR86, or M-tropic HIV-1ADA. In this setting, the enhancing effects of the supernatant on T-tropic and dual-tropic HIV-1 infection and the suppressive effects on M-tropic HIV-1 infection were sustained throughout the 12-d culture period (Fig. 2, A–C).

The effect by continuous treatment with MT-2-conditioned medium on HIV-1 infection of primary CD4⁺ T cells was also investigated in single-round virus replication assays. In this system, the input virus is pseudotyped by Env of interest and expresses the luciferase gene after integration of proviral DNA into the host genome; however, it is unable to complete its life cycle because of the lack of de novo Env production. Therefore, luciferase activity in the infected cell lysates correlates well with the efficiency of virus replication during early events in the viral replicative cycle. As expected, coculture with MT-2 cells in a transwell system before infection (data not shown) or pretreatment with MT-2-conditioned medium (Fig. 3) reduced infectivity of virus pseudotyped by M-tropic Env but increased infectivity of virus pseudotyped by T-tropic HIV-1 Env, suggesting that the steps during the HIV-1 replicative cycle that are influenced by HTLV-I coinfection include early events. Similar results were obtained by using crude

Figure 1. M-tropic HIV-1 infection is downregulated and T-tropic HIV-1 infection is enhanced in primary CD4⁺ T cells cocultured with HTLV-I-infected MT-2 cells in a transwell system. Primary CD4⁺ T cells isolated from HIV/HTLV seronegative individuals either were untreated or were cocultured with MT-2 cells in a transwell (0.2-μm pore membrane) culture for 3 d, and infected with either HIV-1ADA (A) or HIV-1NL4-3 (B). Cell-free supernatants were collected on days 4, 8, and 12 after infection and assayed for RT activity. Experiments were repeated twice with similar results.

Figure 2. M-tropic HIV-1 infection is downregulated and T-tropic HIV-1 infection is enhanced over a 12-d culture period in primary CD4⁺ T cells treated continuously with MT-2 cell culture supernatants. Primary CD4⁺ T cells were pretreated continuously with MT-2 cell culture supernatants. Primary CD4⁺ T cells were pretreated with cell-free crude supernatants from either A3.01 cells (control) or MT-2 cells (MT-2 sup) at a 1:1 ratio for 3 d before infection with HIV-1NL4-3 (A), HIV-1BR86 (B), or HIV-1ADA (C). Culture medium was replaced with the same medium containing either control or MT-2 cell supernatant every 4 d after infection, and RT activity was measured. Representative results from three independent experiments are shown.
supernatants from another HTLV-I producing cell line, HUT-102 (data not shown).

Identification of Negative and Positive Factors Produced by MT-2 Cells. MT-2 cells are known to produce HTLV-I virions, viral proteins such as Tax (37–39), and a number of cytokines (for review see reference 40). Of note, HTLV-I virions, viral proteins such as Tax (37–39), and a number of CC chemokines RANTES, MIP-1α, and MIP-1β, are present in the supernatants from another HTLV-I producing cell line, HUT-102 (data not shown).

HIV-1 infection. Therefore, it is likely that the net effect of crude supernatants from HTLV-I–infected cells on HIV-1 infection of adjacent cells depends upon the balance and/or accumulation of these factors. To clarify which factor(s) is responsible for the positive or negative effects on HIV-1 infection, each component (soluble Tax protein, HIV-suppressive CC chemokines, or HTLV-I virions) was removed from the crude supernatants as described in Materials and Methods. Fig. 4A demonstrates that MT-2 cell supernatants contain solubilized Tax protein (lane 2), which was successfully removed by anti-Tax antiserum followed by protein A/G sepharose treatment (lane 4). MT-2 cell supernatants also contain substantial amounts of the CC chemokines RANTES, MIP-1α, and MIP-1β, which were markedly reduced after treatment with specific antibodies and protein A/G sepharose (Fig. 4B).

CD4+ T cells were treated with these supernatant preparations and infected with NL4-3Luc-R– virus pseudotyped by Env from either HIV-1LAI (T-tropic), HIV-189.6 (dual-tropic), or HIV-1ADA (M-tropic). Supernatants subtracted for the CC chemokines RANTES, MIP-1α, and MIP-1β lost their inhibitory effects on M-tropic HIV-1, and instead enhanced its replication, whereas supernatants subtracted for either Tax or HTLV-I virions retained or further augmented their inhibitory activity. In contrast, subtraction of either component (Tax, CC chemokines, or virions) reduced the ability of the supernatants to increase replication of T- (Fig. 4C) or dual-tropic (data not shown) HIV-1. These results indicate that both soluble Tax protein and HTLV-I virions enhance HIV-1 infection of both M-tropic and T-tropic viral phenotypes, whereas the CC chemokines in the supernatants inhibit M-tropic HIV-1 infection and enhance infection with T-tropic HIV-1 infection. Therefore, supernatants from HTLV-I–infected cells invariably enhance infection with T-tropic HIV-1, since each of the identifiable factors in the supernatants (Tax, CC chemokines, and HTLV-I virions) have a positive effect on replication of viruses of these phenotypes. However, although the effect of the crude supernatant on M-tropic
HIV-1 infection in the experiments shown (Figs. 1 B, 2 C, 3, and 4 C) were inhibitory, the net effect depends on the balance of enhancing and suppressing factors as demonstrated by the subtraction experiments in which individual components of the crude supernatant were removed (Fig. 4 C). In fact, when comparing different donors as sources of CD4+ T cells, we have consistently noted marked enhancement of T-tropic HIV-1 infection. In contrast, the degree of inhibition of M-tropic HIV-1 infection varied greatly among donors, suggesting differences in susceptibility among the donors to the net balance of enhancing and inhibitory factors contained in the MT-2 supernatants.

Soluble Tax Protein Increases Fusogenicity of CD4+ T Cells with Env from HIV-1. The role of HTLV-I Tax protein in HIV-1 infection of CD4+ T cells was further investigated in two different systems. First, we purified Tax protein from E. coli transformed with a GST-Tax fusion protein expression vector (Fig. 5 A). When added to CD4+ T cell cultures, the GST-Tax protein was taken up by the cells and transferred to the nucleus (Fig. 5 B). The effect of Tax protein on HIV-1 infection was tested and compared with that of TCR signaling induced by anti-CD3 antibody. It can be postulated from previous studies that HTLV-I-infected T cells can secrete Tax protein in the concentration (20 ng/ml) used in this study, which is sufficient to produce biological activities such as cytokine production (37–39). Stimulation of CD4+ T cells with purified Tax protein alone or anti-CD3 mAb alone minimally enhanced infectivity of HIV-1 in standard infection assays (data not shown) and single-round virus replication assays; however, these two stimuli in combination markedly enhanced infectivity (Fig. 5 C). Synergy between HTLV-I Tax protein and TCR signaling has also been reported for cytokine production by T cells (43). These results suggest that Tax protein produced by HTLV-I-infected cells is alone a relatively weak enhancer of HIV-1 infection of adjacent cells; however, it synergizes with other inducers of HIV-1 infection.

To determine whether Tax protein is able to influence viral fusion/entry, we performed cell–cell fusion assays. In this system, fusion efficiency between CD4+ T cells treated with purified Tax protein, anti-CD3 antibody, or both, and fusion partner cells expressing HIV-1 Env is measured by b-galactosidase activity in the cell lysates. Similar to the single-round virus replication assays (Fig. 5 C), Tax protein alone or anti-CD3 antibody alone modestly enhanced HIV-1 Env-mediated fusogenic activity of CD4+ T cells, whereas the combination of both markedly enhanced fusogenic activity (Fig. 5 D). These effects are specific to Tax protein in the preparation, since GST protein prepared in the same manner had no effect when used as control and theTax preparation lost its activity after treatment with anti-Tax antiserum followed by protein A/G sepharose (data not shown). These results indicate that the effect of Tax protein on HIV-1 infection is mediated, at least in part, by increasing fusogenic activity.
Induction of Tax Protein in Jurkat Cells Increases Fusogenic Activity with HIV-1 Env. The role of CC chemokines in infection of CD4+ T cells with T-tropic HIV-1 was further investigated by using recombinant CC chemokines. Although stimulation of the cells with anti-CD3 increased replication of T-tropic HIV-1 by 10-fold, CC chemokines alone had no or minimal effect on infectivity of T-tropic HIV-1. In contrast, costimulation of the cells with both anti-CD3 and CC chemokines further increased replication of T-tropic HIV-1 up to an additional threefold (Fig. 7A; data not shown). We have obtained similar results using MIP-1α or MIP-1β (data not shown).

To determine whether CC chemokines are capable of increasing fusogenic activity of the cells with HIV-1 Envs, CD4+ T cells were either untreated or treated with anti-CD3 alone, CC chemokines alone, or both, and then tested for their fusogenicity with cells expressing HIV-1 Env. CC chemokines alone did not increase HIV-1 Env-mediated fusogenic activity, whereas anti-CD3 alone did so modestly. Combination of anti-CD3 and CC chemokines further increased fusogenicity (Fig. 7B). Thus, upregulation of CC chemokines in combination with anti-CD3 antibody, enhances replication of and fusogenicity with Envs from T-tropic HIV-1. (A) Primary CD4+ T cells were either untreated or treated with CC chemokines (200 ng/ml RANTES [R&D Systems]), anti-CD3 antibody, or both for 3 d before infection with HIV-1NL4-3 or HIV-1ADA. Representative results from three independent experiments are shown. (B) Primary CD4+ T cells were treated as in A, and infected with vTF7-3 (fusion targets), and CD4+ T cells stimulated with anti-CD3 were infected with vCB21R as well as rVV expressing HIV-1NL4-3 Env (fusion effectors). Results were means ± SD from three independent experiments.
able to modulate HIV-1 infection of adjacent CD4+ T cells were either untreated or treated with HTLV-I particles (1 μg/ml protein), anti-CD3 antibody, or both for 3 d before infection with NL4-3-Luc-R-E virus pseudotyped by Env from HIV-1_HXB2 or HIV-1ADA. Similar results were obtained twice.

HIV-1 Virions in Combination with Anti-CD3 Enhance HIV-1 Infection. The role of HTLV-I virions in HIV-1 infection of CD4+ T cells was further investigated by using purified HTLV-I particles. Stimulation of the cells with purified HTLV-I particles (1 μg/ml of protein) alone modestly enhanced infectivity of HIV-1 bearing either T- or M-tropic Env in single-round virus replication assays, whereas coinoculation of the cells with both purified HTLV-I particles and anti-CD3 enhanced the infectivity markedly (Fig. 8). These results confirmed a previous study demonstrating that HTLV-I virions are able to enhance HIV-1 replication in CD4+ T cells (25).

In this study we have demonstrated in several different systems that soluble factors from HTLV-I-infected cells are able to modulate HIV-1 infection of adjacent CD4+ T cells in a positive or negative fashion. As previously reported (41), the CC chemokines RANTES, MIP-1α, and MIP-1β produced by HTLV-I-transformed cells suppressed M-tropic HIV-1 infection; however, in our studies, these chemokines were also involved in upregulation of T-tropic HIV-1 replication. We have demonstrated that direct addition of CC chemokines in combination with anti-CD3, to CD4+ T cells renders the cells more fusogenic with HIV-1 Env. Since CC chemokines have been demonstrated to have a variety of effects on T cells (44–50), any of those activities of the CC chemokines may be involved in the enhancement of T-tropic HIV-1 replication. We are currently investigating cellular and molecular mechanisms of the CC chemokine-mediated effect. We have also confirmed a previous study showing that HTLV-I virions are able to activate T cells and enhance T-tropic HIV-1 replication (25).

The mechanisms of Tax-mediated effects may be more complex. HTLV-I Tax protein has been shown to upregulate expression of HIV-1 (8), as well as expression of various cytokines and cytokine receptors involved in T cell activation (for review see references 51, 52), thereby providing favorable circumstances for HIV-1 infection. However, expression of anti-HIV CC chemokines is also induced by direct addition of soluble Tax protein (Moriuchi, H., M. Moriuchi, and A.S. Fauci, unpublished observations). Therefore, the net effect of Tax protein may depend upon the balance or accumulation of those effects. We have demonstrated that Tax protein is able to enhance HIV-1 fusion/entry. It is likely that Tax protein transactivates expression of cellular factors that are required for viral fusion/entry. We have recently cloned the promoter regions for CXCR4 (53) and CCR5 (54), and demonstrated that Tax is able to transactivate these promoters (Moriuchi, H., M. Moriuchi, and A.S. Fauci, unpublished observations). Thus, upregulation of coreceptor expression may be responsible, at least in part, for the effect of Tax on HIV-1 fusion/entry.

Our present study also indicates that the effect of HTLV-I coinfection on the pathogenesis of HIV disease is multifactorial, and that soluble factors produced by HTLV-I-infected cells are capable of enhancing and/or suppressing HIV-1 infection of adjacent cells, depending on the balance of effects of the factors involved and the tropism of the virus. Although an increase in replication of T- and dual-tropic HIV-1 is consistently seen, the balance of enhancing and suppressing factors determines the net effect on M-tropic HIV-1 infection. Discrepancy among previous studies (10–12, 14–17) on the effect of HTLV-I/HIV coinfection on HIV disease progression may reflect these potentially dichotomous effects on M-tropic HIV-1. In this regard, infection with HTLV-I may favor the transition from M- to T-tropic phenotype, which is associated with HIV disease progression (3, 57–59).

In conclusion, this study provides possible mechanisms whereby coinfection of an individual with HIV-1 and HTLV-I influences the course of HIV-1 infection without necessity for actual coinfection of the same cells by the two pathogens. Further studies are required to establish the actual effects of HTLV-I coinfection on the clinical progression of HIV infection in vivo.
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