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

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[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.

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

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).

JPX9 cells and JPX/M cells are 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 CdCl$_2$ for 2 d.

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

Propagation of MT-2–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 kits (R&D Systems). 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 Expressed in Escherichia coli DH5a Strain and HTLV-I Partides 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 dialyzed 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/mg protein (Limulus Amebocyte 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 antiserum (1:2,000), respectively. In some experiments, the GST-Tax preparation was treated by 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 2×10^5 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 Assay. 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]), ADA (M-tropic [reference 30]) or amphotropic murine leukemia virus (AMV), as previously described (31). Approximately 10^5 primary CD4+ T cells were infected with the above luciferase reporter virus (5×10^5 cpm RT activity), and luciferase activity of the cell lysates was measured 4 d after infection using commercially available reagents (Promega, Madison, WI).

Fusion Assay. 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 *lacZ* 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 microtiter plates in the presence of 40 μg/ml of cytosine arabinoside. After 4 h at 37°C, β-galactosidase activity in the cell lysates were 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-
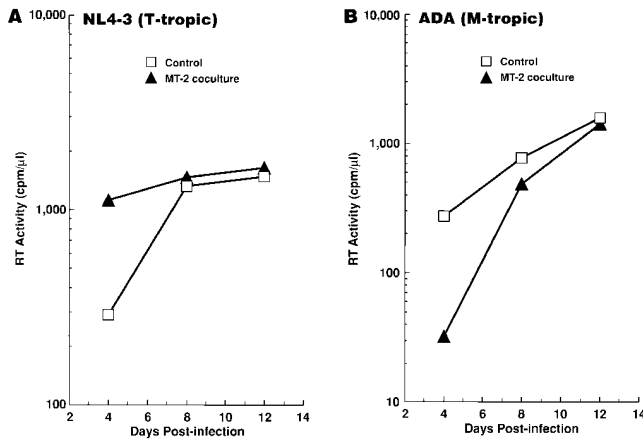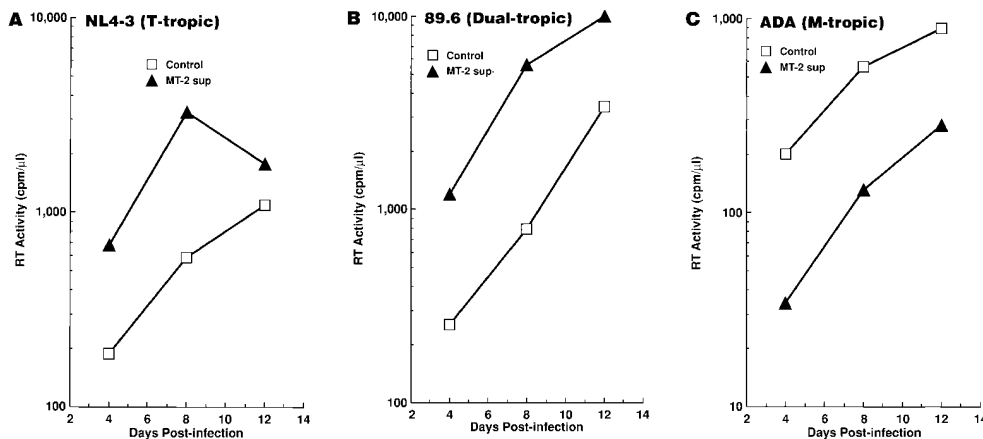
Figure 1. M-tropic HIV-1 infection is downregulated and T-tropic HIV-1 infection is enhanced in primary CD4$^+$ T cells cocultured with HTLV-I–infected MT-2 cells in a transwell system. Primary CD4$^+$ T cells isolated from HIV/HTLV seronegative individuals either were untreated or were cocultured with MT-2 cells in a transwell (0.2-μm pore membrane) culture for 3 d, and infected with either HIV-1$_{NL4-3}$ (*A*) or HIV-1$_{ADA}$ (*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.

out coinfection of the same cell was initially evaluated by coculturing primary CD4$^+$ T cells with MT-2 cells in a transwell system for 3 d before HIV-1 infection. In this system, the two cell populations were separated by a 0.2-μm pore membrane to avoid cell-to-cell contact, which is required for the establishment of infection with highly cell-associated HTLV-I (34–36). Under these conditions, immortalization of CD4$^+$ T cells did not occur and HTLV-I p24 antigen was not detected in the CD4$^+$ T cell culture supernatants over 3 wk (data not shown). Primary CD4$^+$ T cells pretreated in the MT-2 cell coculture system described above were then infected with either M-tropic HIV-1$_{ADA}$ or T-tropic HIV-1$_{NL4-3}$, and RT activity was measured in the infected cell supernatants. CD4$^+$ T cells

cocultured with MT-2 cells before infection produced less HIV-1$_{ADA}$ but more HIV-1$_{NL4-3}$ compared with control CD4$^+$ T cells on day 4 after infection (Fig. 1, *A* and *B*); however, these effects were not observed beyond 8 d after infection.

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-1$_{NL4-3}$, dual-tropic HIV-1$_{89.6}$, or M-tropic HIV-1$_{ADA}$. 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 Envs 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 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 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-1$_{NL4-3}$ (*A*), HIV-1$_{89.6}$ (*B*), or HIV-1$_{ADA}$ (*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.
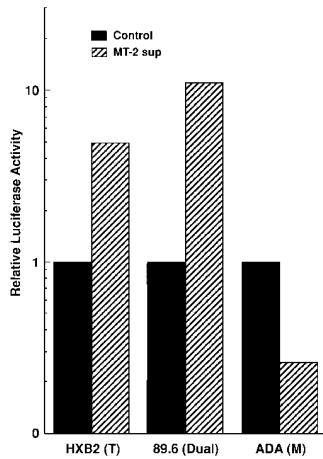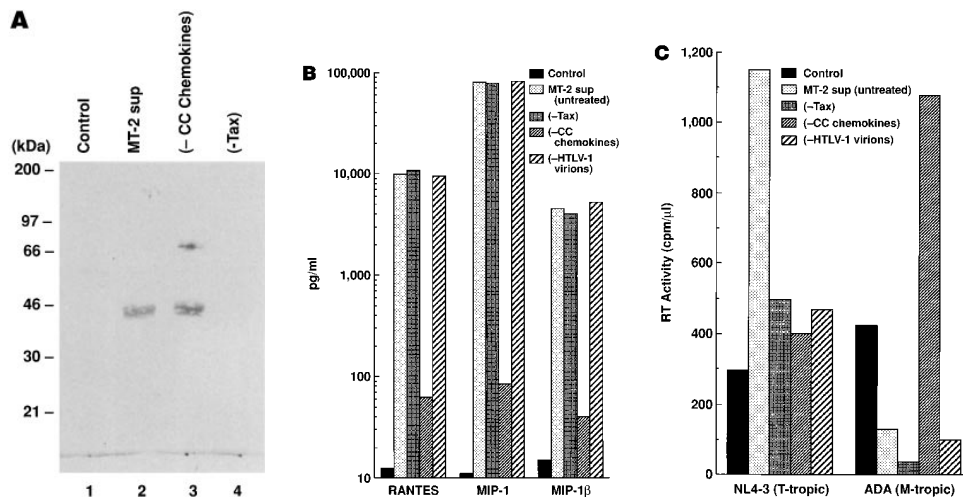
Figure 3. Soluble factors produced by MT-2 cells modulate early events in the HIV-1 replication cycle. Primary CD4+ T cells were pretreated with cell-free crude supernatants from either A3.01 cells (*control*) or MT-2 cells (*MT-2 sup*) at a 1:1 ratio for 3 d before infection with NL4-3-Luc-R$^-$E$^-$ virus pseudotyped by Env from HIV-1$_{HXB2}$, HIV-1$_{89.6}$, or HIV-1$_{ADA}$. Luciferase activity in the infected cell lysates was measured on day 4 after infection. 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–transformed CD8+ T cells were recently shown to produce the CC chemokines RANTES, MIP-1α, and MIP-1β, and to suppress infection of a CD4+ T cell line with M-tropic HIV-1 (41). In contrast, previous studies had demonstrated that mitogenic stimulation induced by HTLV-I virions increased HIV-1 replication (25), and that HTLV-I Tax protein transactivated HIV-1 LTR (8) as well as induced expression of several cytokines including CC chemokines (42). 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 responsi-

ble 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. 4 *A* demonstrates that MT-2 cell supernatants contain soluble 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. 4 *B*).

CD4+ T cells were treated with these supernatant preparations and infected with NL4-3Luc-R$^-$E$^-$ virus pseudotyped by Env from either HIV-1$_{HXB2}$ (T-tropic), HIV-1$_{89.6}$ (dual-tropic), or HIV-1$_{ADA}$ (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. 4 *C*) 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

Figure 4. HTLV-I transactivator Tax protein and the CC chemokines RANTES, MIP-1α, and MIP-1β produced by MT-2 cells modulate HIV-1 infection. (*A*) MT-2 cells produce and secrete a soluble form of HTLV-I transactivator Tax protein. Crude supernatants from A3.01 cells (*control*; lane *1*) or MT-2 cell supernatants treated with control serum (*MT-2 sup*; lane *2*), mAbs to RANTES, MIP-1α, and MIP1β (−*CC chemokines*; lane *3*), or anti-Tax antiserum (*-Tax*; lane *4*) followed by protein A/G sepharose were immunoprecipitated with anti-Tax antiserum and protein A/G sepharose, subjected to 10% SDS-PAGE, and transferred to nitrocellulose membrane for Western blotting. The blot was probed with anti-Tax antiserum and horseradish peroxidase–conjugated protein A (Amersham Corp., Arlington Heights, IL). (*B*) MT-2 cells produce and secrete CC chemokines. Concentrations of RANTES, MIP-1α, and MIP-1β in the above supernatants were measured with ELISA. (*C*) The effect of subtraction of each component from MT-2 cell supernatants on HIV-1 infection. Primary CD4+ T cells were treated with the indicated supernatants at a 1:1 ratio for 3 d before infection with HIV-1$_{NL4-3}$ or HIV-1$_{ADA}$. Culture medium was replaced with the same medium every 4 d after infection, and RT activity was measured. Peak RT activities on day 12 are shown. Experiments were repeated three times with similar results.
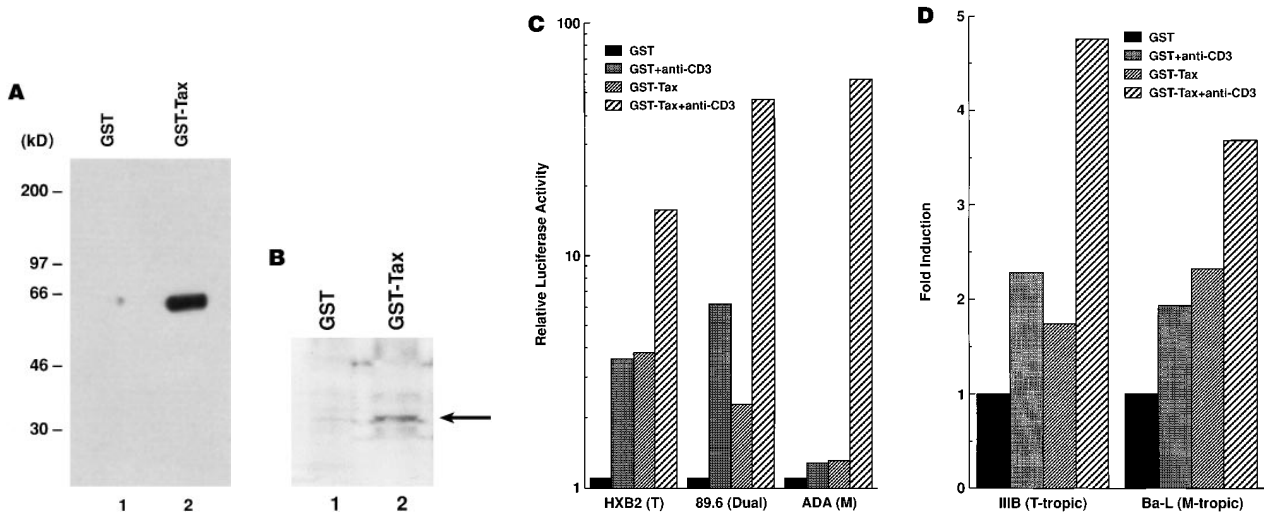
Figure 5. Purified Tax protein, in combination with anti-CD3 antibody, increases susceptibility to fusogenicity with Env from HIV-1. (*A*) GST–Tax fusion protein was purified as described in the Materials and Methods, subjected to 10% SDS-PAGE (lane *2*), and transferred to nitrocellulose membrane for Western blotting, and then the blot was probed with anti-Tax antiserum and horseradish peroxidase–conjugated protein A. GST protein was prepared in the same manner and used as a control (lane *1*). (*B*) GST–Tax fusion protein was taken up by the cells and transferred to the nucleus. Nuclear extracts were prepared from primary CD4$^+$ T cells treated with either GST (100 ng/ml; lane *1*) or GST–Tax (100 ng/ml) (lane *2*), and analyzed in Western blotting as described above. The arrow indicates the position of GST–Tax protein. (*C*) Purified Tax protein in combination with anti-CD3 antibody enhances infectivity of virus pseudotyped by Envs from HIV-1$_{HXB2}$, HIV-1$_{89.6}$, or HIV-1$_{ADA}$. Primary CD4$^+$ T cells were treated with either GST (20 ng/ml) or GST–Tax protein (20 ng/ml) in the presence or absence of anti-CD3 mAb (OKT3 ascites 1:2000) for 3 d before infection. Representative results from four independent experiments are shown. (*D*) Purified Tax protein in combination with anti-CD3 antibody enhances HIV-1 Env-mediated cell-to-cell fusogenic activity. Primary CD4$^+$ T cells were treated as above, infected with vTF7-3 (expressing T7 polymerase), and mixed with BSC-1 cells infected with vCB21R (encoding *lacZ* gene under the control of the T7 promoter) as well as rVV expressing the indicated HIV-1 Env. Fusion index indicates OD$_{570}$ value obtained for HIV-1 Env-mediated cell fusion relative to the background value obtained for nonfusogenic uncleaved Env-mediated cell fusion. Representative results from three independent experiments are shown.

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 Envs 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 β-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 the Tax 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.
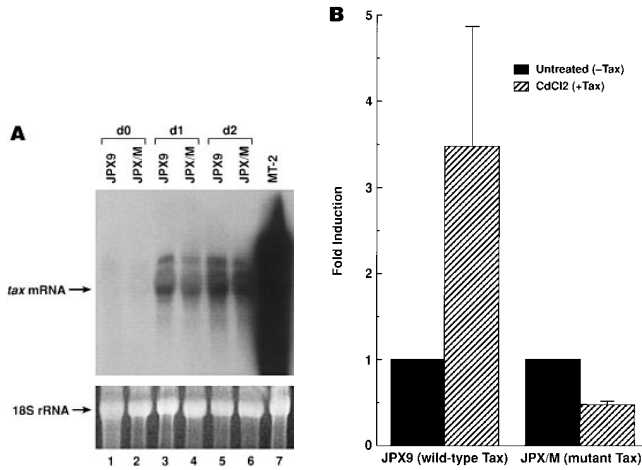
Figure 6. Tax protein expressed in a CD4+ Jurkat T cell line enhances HIV-1 Env-mediated cell-to-cell fusion efficiency. (*A*) *tax* is inducibly expressed in JPX9 cells (Jurkat cells stably transfected with wild-type HTLV-I Tax protein under the control of the metallothionein promoter) and JPX/M cells (Jurkat cells stably transfected with a nonactive mutant form of Tax). JPX9 and JPX/M cells were treated with $CdCl_2$ (10 μM), and total RNA was prepared from cells before treatment (day 0) or on day 1 or 2 after treatment. Total RNA from MT-2 cells was also prepared as a positive control. 15 μg of total RNA was analyzed in Northern blotting using a probe specific to the *tax* gene (*top*). Ethidium bromide staining of the gel is presented (*bottom*), showing the comparable levels of 18S rRNA loaded. (*B*) Fusogenicity of JPX9 cells increases after induction of Tax expression. JPX9 and JPX/M cells were either untreated or treated with $CdCl_2$ for 2 d, and cell–cell fusion assays were performed for the measurement of HIV-1 IIIB Env-mediated fusogenic activity. Fold induction indicates fusogenic activity mediated by Tax (+*Tax*) relative to the baseline (−*Tax*) fusogenic activity of each cell line. Results are means ± SD of four independent experiments.

Induction of Tax Protein in Jurkat Cells Increases Fusogenic Activity with HIV-1 Env.   The effect of Tax protein on HIV-1 infection was investigated in another system. Jurkat cells JPX9 and JPX/M were stably transfected with the

wild-type and mutant form of HTLV-I Tax protein, respectively, and Tax expression was induced by stimulation with $CdCl_2$ (Fig. 6 *A*). Jurkat cells express CXCR4 (a major coreceptor for T-tropic HIV-1), but not CCR5 (a major coreceptor for M-tropic HIV-1), as well as CD4 (a receptor for HIV-1); therefore, T-tropic Env-mediated fusogenic activity was tested in these cells in the presence (+Tax) or absence (−Tax) of $CdCl_2$.

Induction of expression of wild-type Tax protein in JPX9 cells rendered these cells more fusogenic with cells expressing HIV-1 T-tropic IIIB Env, whereas expression of the mutant Tax protein did not increase fusogenicity of JPX/M cells (Fig. 6 *B*). The slight decrease in fusogenic activity of JPX/M cells after $CdCl_2$ treatment is probably due to the toxic effect of Cd^{2+}.

CC Chemokines in Combination with anti-CD3 Enhance Infection and Fusogenicity with Envs from T-tropic HIV-1.   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. 7 *A*; 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. 7 *B*). Thus, upregula-
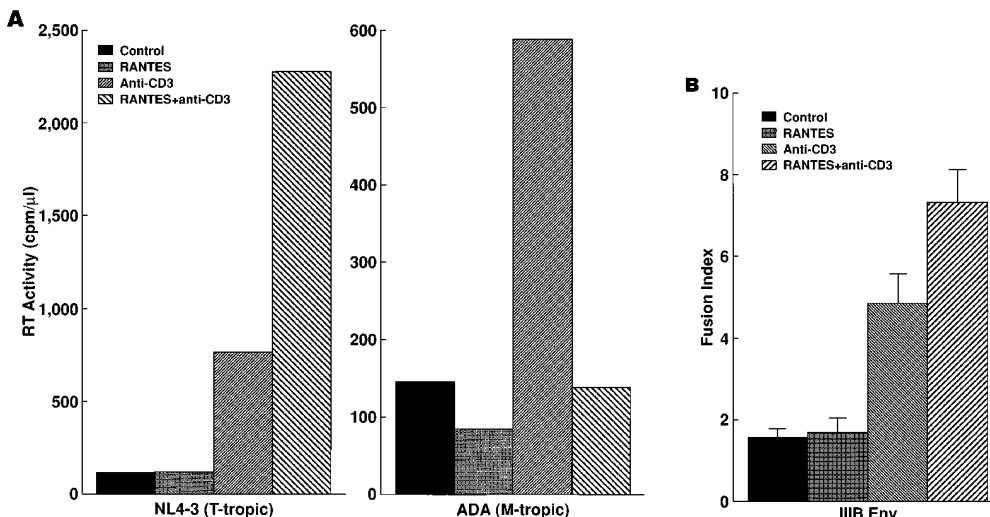
Figure 7. Recombinant CC chemokines, in combination with anti-CD3 antibody, enhance replication of and fusogenicity with Envs from T-tropic HIV-1. (*A*) Primary CD4+ T cells were either untreated or treated with CC chemokines (200 ng/ml RANTES [R&D Systems]), anti-CD3 antibody, or both for 3 d before infection with HIV-1$_{NL4-3}$ or HIV-1$_{ADA}$. 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-1$_{NL4-3}$ Env (fusion effectors). Results were means ± SD from three independent experiments.
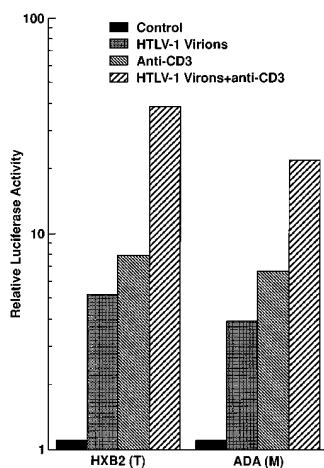
Figure 8. HTLV-I virions, in combination with anti-CD3 antibody, enhance replication of HIV-1. Primary CD4$^+$ T cells were either untreated or treated with HTLV-I particles (1 μg/ml protein), anti-CD3 antibody, or both for 3 d before infection with NL4-3-Luc-R$^-$E$^-$ virus pseudotyped by Env from HIV-1$_{HXB2}$ or HIV-1$_{ADA}$. Similar results were obtained twice.

tion of T-tropic HIV-1 infection by CC chemokines is mediated, at least in part, by enhancement of fusogenic activity of the cells with HIV-1 Envs.

HTVL-I 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 costimulation 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 Envs. 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 in-

vestigating 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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