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HIV Tat Binds Egr Proteins and Enhances Egr-dependent Transactivation of the Fas Ligand Promoter*

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HIV Tat can enhance activation-induced up-regulation of Fas ligand (FasL), which may contribute to T cell apoptosis in human immune deficiency virus (HIV)-infected individuals. We have assessed functional and physical interactions between Tat and the Egr family of transcription factors (Egr-1, -2, and -3), the latter two of which are major participants in activation-induced FasL up-regulation. Here we report that whereas Tat itself has no effect on the FasL promoter, it binds to Egr-2 and -3 and synergizes with them to superinduce expression of a FasL promoter-driven reporter. A Tat molecule containing a single amino acid substitution that results in the loss of transactivation activity for the HIV long terminal repeat still binds Egr-3 but can no longer enhance Egr-mediated transactivation of the FasL promoter. Furthermore, the mutated Tat acts as a dominant negative inhibitor, blocking the superinduction of FasL caused by wild type Tat. Because Tat is present in virus-infected cells and in the serum of HIV-infected individuals, these results suggest that increased expression of FasL in these circumstances may result from the cooperative activities of activation-induced Egrs and Tat.

The regulated apoptosis of peripheral lymphocytes is necessary to maintain a competent and tolerant immune system (1). The predominant signaling pathway involved in achieving this is initiated by the engagement of the "death receptor" Fas (CD95) with its ligand, FasL[1] (2). Because Fas-mediated apoptosis is irreversible it must be tightly regulated. Fas and its downstream signaling machinery are present in most cells, and regulation occurs both pre- and post-Fas engagement (3). For T cells, one of the major levels of control is exerted at the level of FasL expression. FasL mRNA is not expressed in resting T cells but is induced shortly after an activating stimulus (4). The up-regulation of Fas ligand is responsible for activation-induced apoptosis of certain T cell lines, T cell hybridomas, and pre-activated T cells (5). Glucocorticoids, cyclosporin A, FK506, retinoids, and transforming growth factor β prevent activation-induced apoptosis by inhibiting the up-regulation of FasL (6–9).

Many transcription factors have been implicated directly or indirectly in up-regulation of FasL expression, including c-Myc (10), interferon regulatory factors (11), NF-AT (12, 13), NF-κB (14, 15), and AP-1 (16). We have found that the Egr family members Egr-2 and Egr-3, but not the more abundant Egr-1, are essential for activation-induced up-regulation of FasL (17, 18). Egrs are a family of inducible transcription factors that require *de novo* mRNA and protein synthesis to be expressed. Whereas Egr-1 can be induced by phorbol 12-myristate 13-acetate alone (by activating protein kinase C), in T cells an elevation of intracellular Ca^{2+} is also required to induce Egr-2 and -3 (19). This is because Egr-2 and –3 are transcriptionally regulated by NF-AT, as evidenced by the fact that their promoters contain NF-AT binding sites, their induction is inhibited by cyclosporin A, and their expression is impaired in NF-AT_c/NF-AT_p-deficient animals (17, 20). In fact, much of the NF-AT dependence of FasL transcription itself may be secondary to the NF-AT dependence of these important transcriptional regulators. The FasL promoter has an Egr binding site 207–214 bp upstream of the transcriptional initiation site. This site must be intact for activation to induce downstream reporter gene, and transient overexpression of Egr-2 or -3 alone is sufficient to induce FasL mRNA expression in epithelial cell lines (17, 18).

Tat is an HIV-encoded transcriptional activator required for replication of the viral genome (21). The major effect of Tat on HIV gene transcription is to increase the efficiency of elongation after binding to the transactivation response element in viral RNA (22). The association of Tat with the transactivation response element and the complex of Cdk9 and cyclin T facilitate the phosphorylation of the C-terminal domain of RNA polymerase II and therefore enhance elongation (23, 24). The N-terminal portion (amino acids 1–48) of Tat is an activation domain that can function as a transactivator when fused with heterologous DNA- or RNA-binding proteins (25), apparently because of its ability to bind the Cdk9-cyclin T complex. It has also been shown that Tat may exert its action on gene transcription through associated factors such as Tip30 (26). HIV-infected cells can secrete Tat, and many studies have shown that exogenous Tat has a variety of profound effects on different cells. Among the direct biological activities attributed to Tat are increased NF-κB binding to DNA and release of monocyte chemoattractant protein-1 from astrocytes (27), monocyte chemoattraction (28, 29), induction of monocyte-derived IL-1α and TNFα and monocyte activation (30), up-regulation of caspase-8 expression (31), activation of cyclin-dependent kinases (32), and inhibition of major histocompatibility class (MHC) I and β_2-microglobulin transcription (33, 34). Given the evidence that FasL-Fas interactions may account for bystander killing of T cells in patients infected with HIV (35), one of the more intriguing activities ascribed to Tat is that it synergizes with T cell activating stimuli in the up-regulation of FasL expression (36).

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[1] The abbreviations used are: FasL, Fas ligand; LTR, long terminal repeat; FLRE, FasL response element; GST, glutathione *S*-transferase; PBMC, peripheral blood mononuclear cells; HIV, human immunodeficiency virus.

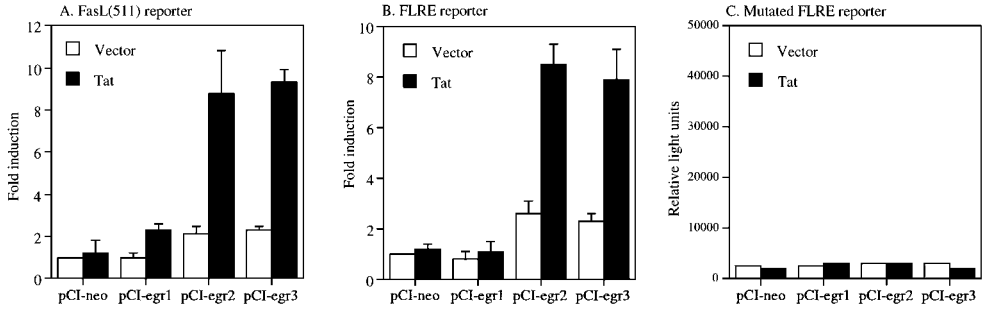

FIG. 1. **HIV Tat enhances transactivation of FasL promoter by Egr-2 and -3.** HeLa cells in 96-well tissue culture clusters were transfected with indicated expression plasmids and luciferase reporters containing the 511-bp promoter of FasL (*A*), FLRE (*B*), or a mutated FLRE that cannot bind Egrs (*C*). The data in *A* represent the mean ± S.E. of five independent experiments, and the results in *B* and *C* represent the mean ± S.E. of three independent experiments.

In this study, we asked if the ability of Tat to synergize with activation to superinduce FasL reflects an interaction, direct or indirect, with Egr family transcription factors. Here we report that Tat physically interacts with all Egr family members and synergizes with Egr-2 and –3 but not Egr-1 to increase the expression of a reporter gene driven by the FasL promoter, an activity that depends upon both an intact Egr binding site in the promoter and the transactivation activity of Tat. Furthermore, a transactivation-deficient form of Tat acts as a dominant negative, abrogating the ability of native Tat to co-activate the FasL promoter. These results provide a molecular mechanism for the ability of Tat to synergistically enhance FasL expression, and they suggest a possible means for interfering with this phenomenon.

EXPERIMENTAL PROCEDURES

Cell Line—Human cervical adenocarcinoma cell line HeLa was cultured in Dulbecco's modified Eagle's medium (BIOSOURCE International, Camarillo, CA) supplemented with 4 mM glutamine, 50 μM 2-mercaptoethanol, 100 units/ml of penicillin, 150 μg/ml of gentamicin, and 10% fetal calf serum.

Plasmids—The luciferase reporter plasmid pGL-3 containing the 16-bp FLRE (Fas ligand response element) or the 511-bp fragment of the human FasL promoter region was constructed as described (17). In the mutated FLRE reporter, four nucleotides (GTGG) at the center of 16-bp FLRE were replaced with CACC (17). The expression plasmids encoding NGFI-A (Egr-1), Egr-2, and Egr-3 have been reported previously (37). For *in vitro* translation, the cDNAs of Egr-1 and -2 were subcloned into pCI-neo using *Sma*I and Mlu/Xba sites, respectively. The cDNA of Egr-3 was inserted into the *Hin*dIII/*Bam*HI site of pSP73. The plasmid pGEX-tat (containing cDNAs encoding Tat 1–72) was kindly provided by Dinah Singer and Jocelyn Weissman (National Cancer Institute, NIH, Bethesda, MD). The constructs pGEX-tat 1–48 and pGEX-tat 49–72 were made by generating corresponding cDNAs using PCR and inserting them into the *Eco*RI/*Not*I site of pGEX-4T-1. To generate other GST-Tat fragments (Tat-(1–20), Tat-(10–30), Tat-(20–40), and Tat-(30–48) double-stranded oligonucleotides encoding the corresponding amino acids were synthesized and cloned into pGEX-4T-1 with *Eco*RI/*Not*I sites. The constructs pGEX-tat K41T (containing cDNA encoding Tat 1–86) has been described previously (38). The expression vectors pCI-Tat and pCI-Tat(K41T) were made by cloning corresponding cDNAs into *Eco*RI/*Not*I sites of pCI-neo.

In Vitro Translation and Binding Assays—The GST fusion proteins were induced by isopropyl-1-thio-β-D-galactopyranoside in *Escherichia coli* DH5α, transformed with the indicated pGEX constructs, and purified with glutathione beads according to the manufacturer's instructions (Amersham Biosciences). *In vitro* translation of Egr-1, Egr-2, and Egr-3 was performed with T7 polymerase and TNT-coupled reticulocyte lysate systems (Promega, Madison, WI) in the presence of [^{35}S]methionine. For binding assays, bead-bound GST fusion proteins were incubated with *in vitro*-translated products at 4 °C for 2 h. After washing four times with Tris-buffered saline (50 mM Tris, pH 7.5, 150 mM NaCl) containing 0.05% Tween 20, the beads were heated at 100 °C for 3 min in 2× SDS loading buffer (100 mM Tris, pH 6.8, 200 mM dithiothreitol, 4% SDS, 0.2% bromphenol blue, 20% glycerol). Proteins were separated on 10% SDS-PAGE and visualized using a Storm PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Transient Transfection and Luciferase Assays—For transfection of HeLa cells, triplicate 200-μl cultures were transfected with 100 ng of the luciferase reporter plasmids and the indicated expression plasmids by the calcium phosphate technique (39). In some experiments, a pSV-β-galactosidase reporter was cotransfected as a control of transfection efficiency. In transfection experiments using 6-well tissue culture cluster, 250 ng of FLRE-luciferase reporter and 250 ng of *egr-3*-expressing constructs were cotransfected with LipofectAMINE2000 (Invitrogen). Luciferase activity was determined as relative fluorescence units using Promega luciferase assay substrates (Promega) and a Monolight 2010 luminometer (Analytical Luminescence Laboratory, San Diego, CA). Error bars in the figures represent the S.E. of the arithmetic means.

Immunoprecipitation and Immunoblotting—For immunoprecipitation, HeLa cells expressing Tat and Egr-1 or -3 were lysed with radioimmune precipitation buffer (10 mM phosphate, pH 7.2, 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% Nonidet P-40, 1% sodium deoxycholate) supplemented with protease inhibitors aprotinin (10 μg/ml), leupeptin (10 μg/ml), and AEBSF (4-(20aminoethyl)benzene sulfonyl fluoride; 1 mM). After centrifugation and removal of the insoluble pellets, the lysates were diluted with phosphate-buffered saline (1:5) and added to protein A beads that have been precoated with anti-Egr-1 or anti-Egr-3 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Following incubation at 4 °C for 2 h the beads were washed with Tris-buffered saline containing 0.05% Tween 20. The bead-bound proteins were then heated at 100 °C for 3 min in 2× SDS loading buffer and separated on 10% SDS-polyacrylamide gel. Immunoblotting was performed as described (40). Briefly, transfected cells were harvested and lysed with radioimmune precipitation buffer. After centrifugation and removal of the insoluble pellets, the lysates were separated by SDS-PAGE (16%) and transferred to a nitrocellulose membrane. Monoclonal anti-Tat antibody (Immuno Diagnostics, Woburn, MA) was used to detect expressed Tat. The blot was visualized with horseradish peroxidase-labeled goat anti-mouse antibody and enhanced chemiluminescence (Amersham Biosciences).

RESULTS

Tat Synergizes with Egr-2 and Egr–3 to Induce the FasL Promoter—Ectopic expression of Egr-2 or Egr-3 but not Egr-1 can induce expression of luciferase reporters driven by the 511-bp sequence of FasL promoter or the 16-bp FLRE (Egr binding site) in the FasL promoter. To determine whether Tat can synergize with Egrs in transactivating the FasL promoter, HeLa cells were cotransfected with a luciferase reporter construct containing the 511-bp FasL promoter and vectors expressing Tat with or without Egr-1, Egr-2, or Egr-3 (Fig. 1*A*). A suboptimal amount of Egr cDNA was used to enhance the detection of synergy, if any, between these transcription factors and Tat. Expression of Tat alone had no effect on reporter activity, and the limiting amounts of Egr-2 or -3 used resulted in only a 2.1- and 2.3-fold induction of the luciferase activity, respectively (Fig. 1*A*). When Egr-2 or Egr-3 and Tat were coexpressed, however, there was an 8- to 10-fold up-regulation of FasL promoter activity. To determine whether Egr binding to its previously identified cognate site in the FasL promoter was involved in this synergy, experiments were performed with a luciferase reporter driven by the 16-bp FLRE alone (Fig. 1*B*).

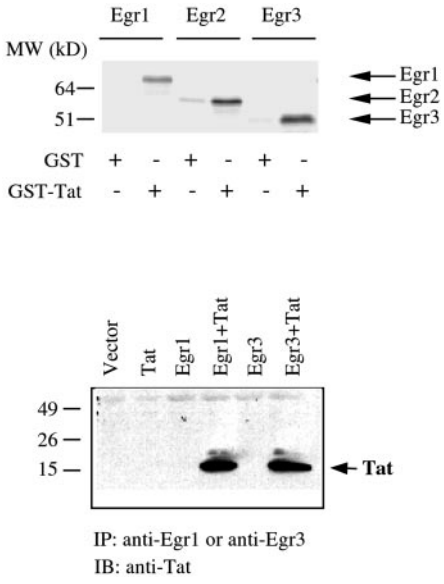

FIG. 2. **HIV Tat binds to Egr family proteins *in vitro* and *in vivo*.** *A*, binding of GST-Tat with Egr-1, -2, and -3. *In vitro*-translated Egr-1, -2, or -3 was incubated with recombinant GST or GST-Tat bound to glutathione beads. After thorough washing, proteins associated with the beads were eluted with 2× gel-loading buffer, separated on 10% SDS-PAGE, and visualized by autoradiography. *B*, association of Tat with Egr-1 and -3 in cells. HeLa cells in 6-well culture clusters were transfected with the indicated vectors encoding Tat (1 μg), Egr-1, and/or Egr-3 (1 μg). After 24 h the cells were harvested, and cell lysates were subjected to immunoprecipitation and immunoblotting. The first four lanes were immunoprecipitated with anti-Egr-1, and the last two lanes were immunoprecipitated with anti-Egr-3.

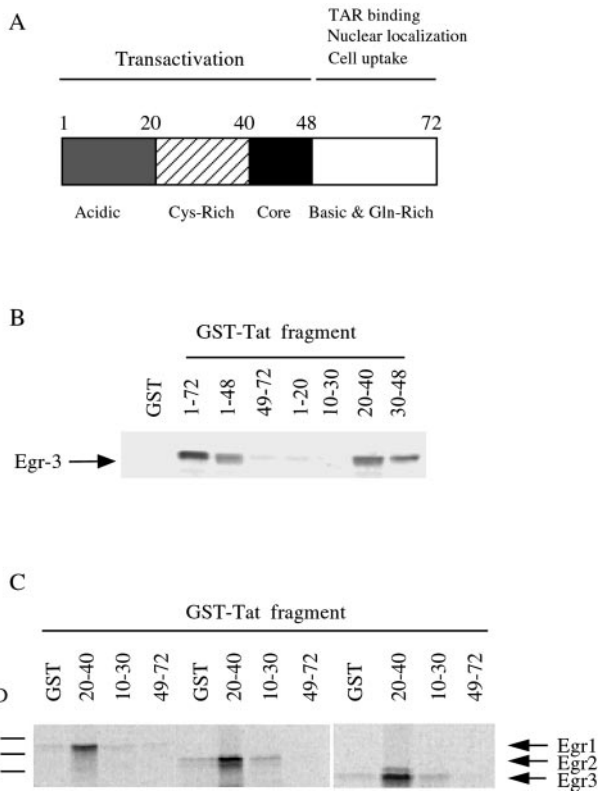

FIG. 3. **Mapping of the region of Tat that interacts with Egrs.** *A*, schematic representation of HIV Tat. *B*, binding of the indicated GST-Tat fragments with *in vitro* translated Egr-3. *In vitro*-translated Egr-3 was incubated with the indicated recombinant GST-Tat fusion proteins bound to glutathione beads. Analysis of binding was performed as described in the legend to Fig. 2. *C*, binding of *in vitro*-translated Egr-1, -2, and -3 with GST-Tat fragments. Binding and analysis was performed as in *B*.

Egr-2 and –3 induced a 2- to 2.5-fold enhancement in reporter activity. Furthermore, similar to the full-length 511-bp FasL promoter, whereas Tat by itself had no effect on luciferase activity, it substantially increased reporter activity when coexpressed with either of these Egr family members.

It has been shown that although Egr-1 binds the FLRE, it is incapable of inducing FLRE (or FasL promoter)-dependent transcriptional activity (17). Therefore, we asked if Tat could also synergize with Egr-1 in FasL induction. As shown in Fig. 1*B*, the combination of Tat and Egr-1 had no effect on the FLRE-driven reporter. This combination also had little effect on a reporter driven by the full 511-bp FasL promoter (Fig. 1*A*). The requirement for direct binding of Egr-2 and –3 binding to the FLRE was tested using a mutated FLRE in which four nucleotide substitutions prevent its interaction with Egr family members. As shown in Fig. 1*C*, this reporter construct was not induced by Egr family members, and the further addition of Tat had no effect. Those results demonstrate that HIV Tat synergizes with Egr-2 and –3 to activate the FasL promoter and that this requires the binding of the Egrs to the FLRE in the FasL promoter.

Tat Binds Egrs—Because Tat enhancement of FasL promoter transcription depends on the concomitant presence of Egr-2 or -3, we asked whether these molecules interact physically. *In vitro*-translated ^{35}S-labeled Egr family proteins were incubated with glutathione beads coated with a GST-Tat fusion protein or with GST alone. After thorough washing, bound proteins were eluted by heating at 100 °C for 3 min in the presence of 2× loading buffer and analyzed by SDS-PAGE and autoradiography. GST protein alone retained little if any of the Egr proteins (Fig. 2*A*), whereas beads coated with GST-Tat protein pulled down ^{35}S-labeled Egr-1, -2, and -3. To determine whether Egrs can bind Tat under more physiologic conditions (*i.e.* in cells), Egr-1 or Egr-3 was cotransfected with Tat into HeLa cells (Fig. 2*B*). When these molecules were coexpressed, anti-Egr-1 (*lane 4*) and anti-Egr-3 (*lane 6*) specifically co-precipitated Tat. Together these results demonstrate that Egrs bind Tat *in vitro* and *in vivo*.

The region of Tat that binds the Egrs was mapped with GST fusion proteins containing subregions of the Tat molecule. Initially, two portions of Tat were analyzed: residues 1–48, essential for the transactivating properties of Tat and for binding to the Cdk9-cyclin T complex (41); and residues 49–72, which contains the basic and glutamine-rich domains of Tat and is involved in transactivation response RNA binding, nuclear localization, and transmembrane transport (25) (Fig. 3*A*). *In vitro*-translated Egr-3 was added to beads coated with similar amounts of GST alone, GST-Tat, GST-Tat-(1–48), or GST-Tat-(49–72) (Fig. 3*B*, *lanes 1–4*). GST-Tat-(1–48) and GST-Tat-(1–72) bound Egr-3, but GST-Tat-(49–72) failed to do so. Furthermore, analysis with GST-Tat fusion proteins containing overlapping fragments of Tat revealed that Tat residues 20–40 and 30–48 bound Egr-3, whereas Tat residues 10–30 did not (Fig. 3*B*, *lanes 5–8*). The binding of GST-Tat fragments with Egr-1 and Egr-2 was also examined. As shown in Fig. 3*C*, both Egr-1 and Egr-2 bound GST-Tat-(20–40) but failed to bind either GST-Tat-(10–30) or GST-Tat-(49–72). Therefore, all three Egrs interact with a region of Tat encompassed by residues 20–40, and this region was further refined to amino acids 30–40 by its binding to Egr-3. Because this region is vital for Tat-mediated gene transactivation, these results suggest that direct interaction between the activation domain of Tat and Egrs is responsible for the superinduction of the FasL promoter.

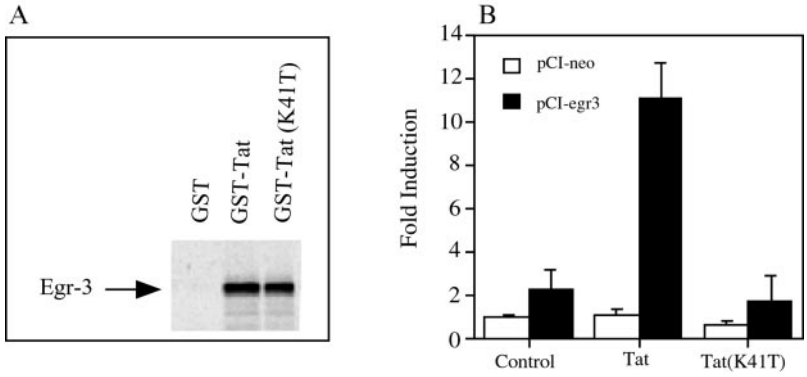

FIG. 4. **Transcriptional activity of Tat is required for its ability to enhance Egr-3-dependent transactivation.** *A*, *in vitro*-translated and ^{35}S-labeled Egr-3 was incubated with indicated GST fusion proteins, and analysis of binding was carried out as described in the Fig. 2 legend. *B*, mutated Tat (*Tat*(*K41T*)) cannot enhance Egr-3-dependent up-regulation of luciferase activity driven by FLRE. The experiments were carried out as described in the legend to Fig. 1.

A Transcriptionally Inactive Tat Mutant (K41T) Inhibits the Effect of Tat on Egr-dependent Transactivation—Substitution of Tat Lys-41 with a threonine residue eliminates its ability to promote transcription of the HIV LTR (42). Because this residue is vital for Tat-induced gene transactivation, we asked whether Tat lacking Lys-41 could still interact with Egr-3 and enhance FLRE-dependent transcription. As predicted by the analysis of the binding of Tat fragments (Fig. 3, *B* and *C*), Tat containing an amino acid substitution at residue 41 was similar to wild type Tat in its ability to bind Egr-3 (Fig. 4*A*). However, unlike wild type Tat, Tat(K41T) was unable to up-regulate Egr-3-dependent induction of a FLRE-driven luciferase (Fig. 4*B*). Thus, the capacity of Tat to transactivate is required for synergism with Egrs.

The fact that Tat(K41T) was able to bind Egr-3 but unable to superinduce gene transcription raised the possibility that this mutant might act as a dominant negative inhibitor of wild type Tat. This was tested by determining whether expression of Tat(K41T) could affect the superinduction of a FLRE-driven reporter by wild type Tat and Egr-3. As shown in Fig. 5*A*, wild type Tat but not Tat(K41T) synergized with Egr-3 in the enhancement of luciferase activity. Moreover, expression of pCI-Tat(K41T) prevented the superinduction of luciferase activity. This was not due to any effect on the expression of wild type Tat, because the levels of Tat were the same whether or not Tat(K41T) was coexpressed (Fig. 5*B*). The Tat K41T migrates more slowly than the wild type Tat because it contains an additional 14 amino acids, which do not otherwise affect the synergy between Tat and Egrs (data not shown). Taken together, these data argue that binding of transactivation-competent HIV Tat to the biologically active Egrs (Egr-2 and –3) is necessary for synergism between these two transcriptional regulators at induction of the FasL promoter.

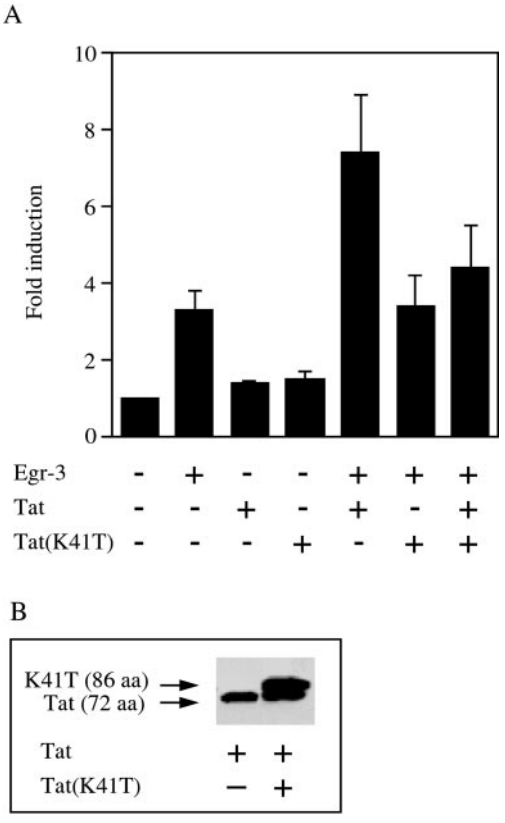

FIG. 5. **Transcriptionally inactive Tat(K41T) inhibits the synergism of Tat with Egr-3.** *A*, HeLa cells were transfected with pGL3-FLRE and the indicated constructs, and 24 h later the cells were lysed and analyzed for luciferase activity. The data represent the mean ± S.E. of six independent experiments. *B*, Western blot for Tat from cells transfected with wild type Tat-(1–72) and Tat(K41T)-(1–86).

DISCUSSION

Depletion of CD4$^+$ T cells is a hallmark of HIV infection. It appears that multiple mechanisms are responsible for the depletion of T cells (43). Given the critical importance of Fas and FasL in regulating the homeostasis of peripheral T cells, many studies have been carried out to investigate potential roles in HIV-induced T cell death (35). It has been shown that peripheral blood mononuclear cells (PBMC) from HIV-infected individuals express higher levels of Fas and are more susceptible to Fas-mediated apoptosis (44, 45). Increased levels of FasL have also been detected in plasma and PBMC from HIV-infected individuals (46–49). The extent of increased expression of FasL on PBMC correlates with disease progression, being greater in those with relatively low CD4$^+$ T cell counts (<200 cells/ml) (50). Furthermore, the higher level of FasL expression on PBMC from HIV-infected children was reduced by anti-retroviral therapy (51). These results suggest that up-regulation of FasL may contribute to the depletion of T cells in HIV-infected individuals.

The means by which HIV infection leads to increase of FasL expression is controversial. *In vitro*, cross-linking of CD4 up-regulates Fas and FasL on PBMC and induces cell death, which can be prevented by F(ab′)$_2$ fragments of anti-Fas antibodies (52). Given that HIV gp120 can bind CD4, this suggested a possible mechanism for up-regulation of Fas and FasL in HIV-infected individuals. However, studies using transformed T cell lines and purified normal CD4$^+$ T cells found that the expression of Fas or FasL was not increased following acute HIV infection *in vitro* (53–56). Furthermore, CD4$^+$ T cells from individuals with genetic defects in Fas expression or signaling were killed normally after HIV infection (56), indicating that Fas and FasL do not participate in T cell death induced by

acute HIV infection. It seems that some of the discrepancies are caused by the presence or absence of monocytes. Unlike the situation with T cells, HIV infection of monocytes/macrophages resulted in the up-regulation of FasL, and these infected cells could kill cocultured Fas^+ T cells (57). The increased expression of FasL on monocytes was also observed following CD4 cross-linking, and removal of monocytes from PBMC abrogated T cell apoptosis following CD4 cross-linking (58). The relevance of these observations to cell death induced by acute HIV infection was further addressed using an HIV strain that also expressed green fluorescent protein in infected cells (68). Whereas infection of purified lymphocytes induced apoptosis mainly in infected cells, infection in the presence of monocytes caused deaths of uninfected T cells as well. Therefore, it is possible that Fas-FasL interactions contribute to depletion of T cells during HIV infection *in vivo*. Consistent with this theory, lymph nodes from HIV-infected individuals have higher levels of FasL, which is mainly expressed by macrophages (59).

Tat enhances transcription of the HIV LTR through association with the Cdk9-cyclin T complex, which in turn phosphorylates the C-terminal domain of the large subunit of RNA polymerase II. If Tat can associate with Egrs and the Cdk9-cyclin T complex at the same time, it is possible that the ability of the Cdk9-cyclin T complex to phosphorylate the C-terminal domain of RNA polymerase II is responsible for the superinduction of FLRE-driven luciferase. Alternatively, the Tat-associated protein Tip30, which has intrinsic kinase activity and can also phosphorylate the C-terminal domain of RNA polymerase II, may participate in the enhancement of transactivation. The region in Tat that is sufficient for association with Egr-3 appears to be residues 30–40. Coincidentally, it has been found that recombinant Tat-(21–40) alone has multiple activities such as induction of cytopathic changes, transactivation of HIV LTR, and activation of NF-κB (60). It is an interesting speculation that those activities are related to its association with Egr family members.

Because lymphocytes from HIV-infected individuals often express activation markers such as HLA-DR, CD45R0, and CD38, it seems likely that up-regulation of FasL in HIV-infected individuals is at least in part the consequence of activation. Given the fact that Tat can be secreted by infected cells and detected in serum from HIV-infected individuals and can cross the plasma membrane of uninfected cells, the observation that exogenous Tat is able to enhance the elevation of FasL mRNA following T cell activation or CD4 cross-linking *in vitro* suggests that Tat contributes to the up-regulation of FasL *in vivo*. Tat can enhance NF-κB activation via induction of oxidative stress and down-regulation of Mn^{2+}-dependent superoxide dismutase expression in T cells (61), suggesting that activation of NF-κB may mediate the synergistic action of T cell activation and HIV Tat in the up-regulation of FasL. This was supported by the finding that NF-κB sites were required for the Tat-mediated increase of transactivation through FasL promoter (62). The data presented in this report demonstrate a different mechanism for Tat enhancement of FasL expression: synergism with activation-induced Egr-2 and -3. T cell activation and increased FasL expression may also result from virus infection directly. For example, HIV-encoded Nef activates T cells (63–65) presumably through direct interaction with the ζ chain of the T cell antigen receptor (66). Simian immunodeficiency virus (SIV) and the HIV-encoded protein Nef have been found to be required for induction of FasL and apoptosis of infected T cells (66, 67). Therefore, it is conceivable that Nef induces the Egr family proteins in infected cells and together with Tat up-regulates FasL following HIV infection. If so, interfering with the Tat-Egr interaction might reduce FasL expression and the secondary depletion of T cells during HIV infection.

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