Cutting Edge: Activation of HIV-1 Transcription by the MHC Class II Transactivator

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*J Immunol* 2000; 164:3941-3945; doi: 10.4049/jimmunol.164.8.3941
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Both macrophages and activated CD4+ T cells can be productively infected by HIV-1, and both cell types express MHC class II molecules. Expression of MHC class II proteins in these cells is regulated by a specific transcriptional coactivator, the class II transactivator (CIITA). In this study, we report for the first time that CIITA expression profoundly influences HIV-1 replication. Stable expression of CIITA in Jurkat cells markedly increased 1) HIV-1 replication as assessed by the p24 Ag production and 2) luciferase expression after transfection with full-length provirus or long terminal repeat constructs. Similarly, transient expression of CIITA increased provirus expression as well as long terminal repeat promoter activity in 293 and HeLa-T4 cells. In contrast, mutant forms of CIITA did not increase HIV-1 expression. This study shows that expression of CIITA increases HIV-1 replication through a transcriptional mechanism. The Journal of Immunology, 2000, 164: 3941–3945.

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Transcription of HIV-1 is dependent on host transcription factors binding to an array of cis-acting DNA elements in the 5′ long terminal repeat (LTR)1 (1–4). In addition to these protein-DNA interactions, most RNA polymerase II promoters are also regulated by proteins that do not directly contact DNA. These so-called coactivator proteins are recruited to the promoter through protein-protein interactions and function as integrators in the assembly of a stable transcription complex (5, 6). Recent evidence suggests that the cellular coactivators p300 and cAMP response element binding protein (CREB) binding protein (CBP) activate HIV-1 transcription through interaction with transcriptional activator, Tat (7–10). However, there is little information concerning the role of cellular coactivator in the Tat-independent activation of HIV-1 transcription.

MHC class II gene expression is activated by a specific transcriptional coactivator protein, the class II transactivator (CIITA), which directs the assembly of a stable transcription complex on class II promoters (11–13). CIITA is believed to activate promoters for MHC class II proteins, MHC class I proteins, and other proteins involved in Ag presentation such as DM and invariant chain (14–18). CIITA is a 124-kDa protein originally identified by Steinle et al. (19). It has an acidic N-terminal activation domain and a C-terminal domain important for protein-protein interactions (20–22). The CIITA protein does not bind directly to class II promoter elements, but interacts with sequence-specific X-box DNA-binding protein RFX5 in a yeast two-hybrid system (23). Other reports have suggested that nuclear factor-Y (NF-Y) binds to RFX to stabilize the interaction between CIITA and RFX (11, 12, 24, 25). CIITA also recruits TFIIID to the class II promoter through interactions with TAFII32 (26). Recently, CIITA was shown to functionally interact with CBP, a transcriptional integrator that increases MHC class II transcription (27, 28). CBP is thought to activate gene transcription by changing promoter accessibility through its intrinsic histone acetyltransferase activity (29, 30).

Recently, we observed that MHC class II Ag HLA-DR expression in T cell lines correlated with higher HIV-1 expression (our unpublished data). In these cells, higher virus expression correlated with increased HIV-1 promoter activity, suggesting a transcriptional mechanism. Because MHC class II gene expression correlated with HIV-1 expression and because class II expression is controlled by CIITA, it seemed possible that CIITA might activate HIV-1 gene transcription in activated T cells and macrophages. In this study, we demonstrate the functional role of CIITA in the activation of HIV-1 expression and replication in several types of cells and suggest a novel mechanism by which HIV expression is regulated in target cells.

Materials and Methods

Cells and viruses

The Jurkat (TIB 152) and H9 (HTB 176) T cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). All T cell lines were cultured in RPMI 1640 medium (BioWhittaker, Walkersville, MD) supplemented with 10% heat inactivated FBS (HyClone, Logan, UT).

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The HeLa cells expressing CD4 (HeLa-T4) and the 293 human embryonic kidney cells were obtained from the National Institutes of Health AIDS Research and Reference Reagents Program (Rockville, MD) and were cultured in DMEM (BioWhittaker) supplemented with 10% FBS. HeLa-T4 cells were maintained in the presence of 500 μg/ml of G418 (Calbiochem, La Jolla, CA).

Cell-free virus was obtained from H9 cells infected with the HIV-1MN strain (National Institutes of Health AIDS Research and Reference Reagent Program). The amount of virus was quantified by measuring p24 Ag by ELISA (Coulter, Hialeah, FL).

Expression plasmids

The pNL4–3.Luc.E receptors (pNL4–3Luc.E), an env– HIV-1 provirus that expresses firefly luciferase, was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. The HIV-1 LTR-Luc construct was obtained from Andrea Cara (National Cancer Institute, National Institutes of Health, Bethesda, MD). The pRL-TK-Luc construct, which expresses Renilla luciferase and is driven by the herpes simplex virus thymidine kinase (TK) promoter, was purchased from Promega (Madison, WI). The human MHC class II transactivator, CIITA cDNA (pcDNA-CIITA) and its mutants, p3FGCITA8 STOP3 and pcDNA3 GTP2del constructs, were prepared as described (16, 20, 31), and the KEBS-NLS-L335 mutant was described (32) and obtained from Dr. Victor Steinme (Hans-Spermann Laboratories, Max-Planck-Institut fur Immunbiologie, Freiburg, Germany). The p3FGCITA8 STOP3 mutant lacks C-terminal 41 aa (aa 1090–1130), the pcDNA3GTP2del lacks aa 461–464 (DAYG), and the KEBS-NLS-L335 mutant contains complete deletion of N-terminal acidic and proline-serine/threonine-rich region (aa 1–334). All three mutants express in the cells but unable to transactivate class II promoter; however, they do exhibit a transdominant negative effect when coexpressed with wild-type CIITA (16, 20, 32). Plasmid DNA was isolated by CsCl density gradient ultracentrifugation.

Generation of stable CIITA transfectants of Jurkat cells

To create a stable cell line that constitutively expresses CIITA, 10^7 Jurkat cells were transfected with 25 μg of CIITA cDNA plasmid (pcDNA-CIITA) and 1 μg of neomycin cDNA by electroporation (300 V, 960 μF) using the Gene Pulser Transfection Apparatus (Bio-Rad, Richmond, CA) according to the manufacturer’s protocol. The CIITA-positive cells (Jurkat-CIITA) were selected by culturing the transfected cells for 3 wk in the presence of 1 mg/ml G418 (Calbiochem) and then maintained at 500 μg/ml G418. Surface expression of HLA-DR was induced by CIITA was confirmed by flow cytometry as described (33).

Transient transfection and reporter assays

Cells were transiently transfected with various combinations of pNL4–3 Luc.E, HIV-1 LTR-Luc, CIITA cDNA, pcDNA vector, and mutant CIITA constructs using DMRIE-C or Lipofectamine reagent (Life Technologies, Grand Island, NY). For Jurkat and Jurkat-CIITA cells, 250 ng to 2 μg pNL4–3 Luc.E or 1 μg HIV-1 LTR-Luc DNA was incubated with 3 μl of DMRIE-C reagent in 500 μl serum-free Opti-MEM (Life Technologies) for 45 min at room temperature. Cells (1 × 10^6) were pretreated for 2–4 h with Opti-MEM before the DNA mixture was incubated with the cell pellet for 2–3 h at 37°C. Cells were then cultured in 48-well plates (2 × 10^5/well) in RPMI 1640 medium with 2% FBS. DNA complexes were similarly prepared for 293 or HeLa-T4 cells by incubating 125–500 ng pNL4–3 Luc.E or 500 ng HIV-1 LTR-Luc DNA in the presence or absence of 0.5–5 μg of CIITA cDNA, pcDNA vector, or CIITA mutant constructs with 6 μl Lipofectamine reagent in 500 μl Opti-MEM. Cells were cultured for 24 h in 48-well plates, preincubated with Opti-MEM, and then DNA mixtures were added to the cells (100 μl/well) for 2–3 h before cells were cultured in DMEM with 1% FBS. After 48–72 h of transfection, cells were harvested, and HIV-1 expression was determined in cell lysates using the Luciferase Assay (Promega). In some experiments, cells were cotransfected with pNL4–3 Luc.E, pRL-TK-Luc (Renilla luciferase) and CIITA expression plasmids and analyzed by the Dual-Luciferase Reporter (Promega) to assess the specific effect of CIITA on HIV-1 expression.

HIV-1 infection of cells

The Jurkat or Jurkat-CIITA cells were infected with HIV-1MN, produced in H9 cells by incubating cell pellets (1 × 10^6) with virus (moi 0.001) at 37°C for 2 h. Cells were then washed and cultured in 48-well plates (5 × 10^5 cells/well, Costar, Cambridge, MA). Six days after infection, supernatants were analyzed for the level of HIV-1 replication by p24 Ag ELISA.

FIGURE 1. Effect of CIITA on HIV-1 expression in Jurkat T cells. A. Jurkat T cells and Jurkat cells expressing CIITA (Jurkat-CIITA) were infected with HIV-1MN and cultured for 6 days. Virus replication was determined by measuring p24 Ag levels in culture supernatants. B. Jurkat and Jurkat-CIITA cells were transfected with 0.25–2.0 μg DNA of the noninfectious pNL4–3 Luc.E construct. After 72 h, HIV-1 expression was detected by measuring the luciferase activity in cell lysates. The results are shown as mean ± SE of three separate experiments (some of the SE bars were too small to see).

Results

CIITA increases HIV-1 expression in Jurkat T cells

To determine whether CIITA can affect HIV-1 expression, we created a stable Jurkat T cell line (Jurkat-CIITA) that constitutively expresses CIITA by transfection of the CIITA expression plasmid. As expected, expression of CIITA in Jurkat cells induced high levels of HIV-1DR expression (99% positive). Untransfected Jurkat cells do not express HLA-DR. Expression of other surface proteins including CD3, CD4, CD25, CD28, and CXCR4 remained essentially unchanged after transfection (data not shown).

To assess the effect of CIITA expression on HIV-1 replication, Jurkat and Jurkat-CIITA cells were infected with HIV-1MN virus. As shown in Fig. 1A, HIV-1MN replicated to higher levels in Jurkat-CIITA than in non-CIITA expressing Jurkat cells with more than 5-fold greater p24 Ag detected in Jurkat-CIITA cells 6 days after infection. These data indicate that expression of the transactivator CIITA can increase HIV-1 replication.

To determine whether expression of CIITA increases virus transcription, Jurkat and Jurkat-CIITA cells were transiently transfected with an env– HIV-1 provirus construct, pNL4–3 Luc.E, which contains a firefly luciferase gene inserted in place of the nef gene. Introduction of this provirus construct into cells results in the production of luciferase under the control of the HIV-1 promoter.
Jurkat-CIITA cells expressed markedly higher levels of luciferase than Jurkat cells at all concentrations of transfected pNL4–3LucE DNA (Fig. 1B). For example, transfection of 2.0 \( \mu g \) pNL4–3LucE DNA resulted in 17-fold higher luciferase expression in Jurkat-CIITA cells than in Jurkat cells. Luciferase expression was also higher in Jurkat-CIITA cells transfected with 0.25 \( \mu g \) plasmid DNA than in Jurkat cells transfected with 2.0 \( \mu g \) of DNA, indicating that expression of CIITA in Jurkat cells increased HIV-1 transcriptional activity.

**CIITA increases HIV-1 expression in multiple cell types**

To determine whether CIITA could also increase HIV-1 transcriptional activity in other cell types, 293 and HeLa-T4 cells were cotransfected with increasing amounts of the CIITA expression plasmid (pcDNA-CIITA) or vector DNA (pcDNA) along with a constant amount of pNL4–3LucE DNA. Transfection of the CIITA plasmid increased luciferase expression up to 29-fold in 293 cells and up to 25-fold in HeLa-T4 cells, whereas transfection of the pcDNA vector plasmid did not increase luciferase expression (Fig. 2A). In control experiments, 293 cells were also transfected with equal amounts of either CIITA or three different nonfunctional CIITA mutants. Wild-type CIITA enhanced HIV-1 expression, while none of the CIITA mutants increased virus expression (Fig. 2B).

To determine whether the CIITA effect was specific for the HIV-1 LTR promoter in our system, transfaction experiments were performed with HeLa-T4 cells where pNL4–3LucE was cotransfected with the CIITA plasmid and the pRL-TK Luc plasmid, which expresses Renilla luciferase driven by TK promoter. As shown in Fig. 2C, while transfection of CIITA plasmid into HeLa-T4 cells significantly increased luciferase expression driven by the HIV-1 LTR, CIITA had no effect on luciferase expression driven by the TK promoter. Transfection of pcDNA vector plasmid did not increase the luciferase expression driven by either the HIV-1 promoter or the TK promoter (data not shown). Taken together, these results indicate that CIITA can increase HIV-1 expression specifically from the LTR promoter in multiple cell types.

**CIITA directly activates the HIV-1 LTR**

The pNL4–3LucE provirus construct used in the above experiments contained intact coding regions for HIV-1 transactivator Tat and several other accessory proteins that may have been required for the effect of CIITA on HIV-1 expression. To determine whether the effect was mediated directly by the HIV-1 LTR, or required HIV-encoded accessory proteins, an HIV-1 LTR-Luc construct, which contains the HIV-1 LTR linked to the luciferase gene, was transfected into Jurkat and Jurkat-CIITA cells, or cotransfected along with the CIITA expression plasmid into HeLa-T4 cells. Expression of CIITA increased luciferase activity from the HIV-1 LTR by 7-fold in Jurkat cells which was comparable to that of the provirus (Fig. 3A), and by 20-fold in HeLa-T4 cells compared with 31-fold by provirus (Fig. 3B). Transfection of pcDNA vector increased neither provirus nor LTR activity (Fig. 3B). These results indicate that CIITA can directly activate the HIV-1 LTR in the absence of HIV-1-encoded accessory proteins.

**Discussion**

The observation that HIV-1 expression and replication is substantially higher in HLA-DR-positive T cell lines than HLA-DR negative cells led us to hypothesize that intracellular CIITA may enhance HIV-1 expression. We found that both stable and transient expression of CIITA enhanced the expression of HIV-1 provirus and LTR. These findings are significant because activated CD4+ T cells and macrophages, the two major cell types infected by HIV-1
The results are shown as mean fold increase. Luciferase activity was measured in cell lysates 3 days after transfection. Although RFX sites in the HIV-1 LTR have not been reported, ITA into the class II promoter (23, 38, 39) bind to sites in the LTR. The nature of that interaction is yet to be determined. It is possible that RFX and NF-Y proteins important for recruiting CIITA to the HIV-1 promoter element(s). How ever, the nature of that interaction is yet to be determined. It is possible that RFX and NF-Y proteins important for recruiting CIITA to the class II promoter (23, 38, 39) bind to sites in the LTR. Although RFX sites in the HIV-1 LTR have not been reported, database sequence analysis identified a putative NF-Y site within HIV-1 LTR (40, 41).

Recently, the X2-box DNA binding protein of CIITA-responsive promoters was identified as the CREB (42), and the CIITA protein was shown to recruit CBP to increase expression of class II genes (27, 28). Although the mechanism of CIITA binding to the HIV-1 promoter is not known, CREB has been reported to bind HIV-1 LTR through AP-1 binding sites (43, 44), and CBP interacts directly with CREB (29, 45) and CIITA (27, 28). Thus, CIITA could be recruited to the HIV-1 promoter via a CBP-CREB complex. CBP is also known to interact with general transcription factors including TATA binding protein (46, 47) and TFII B (29) and facilitate chromatin remodeling and activation of cellular transcription through its intrinsic histone acetyltransferase activity (29, 30). Chromatin remodeling has recently been shown to be an important mechanism in the activation of latent HIV-1 expression (3, 48–50).

Interestingly, CIITA activated the HIV-1 LTR both in the presence and absence of the viral transactivator Tat, demonstrating CIITA can function independently of Tat or other viral accessory proteins. Thus, CIITA may play a role in the early stages of HIV infection or in the activation from latency in memory T cells. In conclusion, we demonstrated for the first time that the class II transactivator CIITA increases HIV-1 replication through transcriptional activation of the LTR promoter. Understanding the mechanism of CIITA-mediated HIV-1 expression could provide a new target for developing therapeutics or preventive measures against HIV or other viral infections.

**Acknowledgments**

We thank Dr. Mark Peeples for his constructive criticism.

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