Virology **296,** 77–83 (2002)
doi:10.1006/viro.2001.1397

Differential Regulation of HIV-1 Clade-Specific B, C, and E Long Terminal Repeats by NF-κB and the Tat Transactivator

Philippe Roof,* Maria Ricci,* Pierre Genin,* Monty A. Montano,†‡ Max Essex,† Mark A. Wainberg,* Anne Gatignol,* and John Hiscott*[,1]

*McGill AIDS Center, Lady Davis Institute for Medical Research, and Departments of Microbiology, Immunology, and Medicine, McGill University, Montréal, Québec H3T 1E2, Canada; and †Harvard AIDS Institute, Harvard School of Public Health, Boston, Massachusetts 02215
‡Dept. of Medicine, Section of Infectious Diseases, Boston University 02215

Received August 29, 2001; accepted January 18, 2002

The major group of human immunodeficiency viruses (HIV-1) that comprise the current global pandemic have diversified during their worldwide spread and may be divided into at least 10 distinct subtypes or clades, A through J. Subtype B predominates in North America and Europe, subtype E predominates in Southeast Asia, and subtype C predominates in sub-Saharan Africa. Functional distinctions in long terminal repeat (LTR) architecture among HIV subtypes have been identified, thus raising the possibility that regulatory divergence among the subtypes of HIV-1 has occurred. In addition to the transcriptional specificity of the HIV-1 LTR, productive HIV-1 replication is also dependent upon the viral Tat protein. Therefore, we sought to investigate whether interactions between host signaling pathways and the NF-κB regions of different HIV-1 subtypes, together with subtype-specific interactions between Tat, TAR, and cellular proteins, modulate the efficiency of HIV-1 clade-specific gene transcription. We demonstrate that the NF-κB sites of subtypes B and E both bind NF-κB-related complexes. However, the duplicated κB sites of the C subtype do not compete for NF-κB binding. Also, clade E Tat protein possesses the highest transactivation capacity, regardless of the LTR context. Furthermore, preliminary evidence suggests that the acetylation of subtype-specific Tat proteins may correlate with their transactivation efficiency.

Key Words: HIV-1 LTR; gene transcription; Tat; NF-κB.

INTRODUCTION

The major group of human immunodeficiency viruses (HIV-1) that comprise the current global pandemic have diversified during their worldwide spread. These isolates can be grouped according to genomic sequences and divided into at least 10 distinct subtypes or clades (Korber *et al.,* 1995). Isolates from different subtypes may differ by 30–40% in amino acid sequence in the Env region, whereas variation ranges from 5 to 20% within a particular subtype (Takehisa *et al.,* 1999). The different subtypes are not distributed evenly throughout the world: subtype B predominates in North America and Europe, subtype E predominates in southeast Asia, and subtype C predominates in sub-Saharan Africa (Zimmerman *et al.,* 1997). Analysis of the current worldwide distribution of HIV-1 subtypes has concluded that HIV-1E and HIV-1C are the most prevalent HIV subtypes in the world (Montano *et al.,* 1997). At this stage of the AIDS pandemic, these subtypes are expanding faster and are of greater global significance than the HIV-1B subtype prevalent in North America and Europe (Montano *et al.,* 1997). The relationship between virus subtype, biological properties, and pathogenicity is unknown, in part because virus replication studies have been performed almost exclusively with subtype B viruses (Jeeninga *et al.,* 2000).

Recombinations are known to occur between subtype groups generating mosaic genomes (Carr *et al.,* 1998; Gao *et al.,* 1998; Robertson *et al.,* 1995). Although currently no evidence exists for specific subtype variations affecting virulence or transmission, controversial data have suggested that subtype E viruses exhibit high replication rates and virulence in Langerhans cells (Soto-Ramirez *et al.,* 1996). However, the observations of Pope *et al.* (1997) do not support the conclusion that subtype E strains have a preferential tropism for dendritic cells and indicate that other explanations for the rapid heterosexual spread of subtype E strains in Asia need to be considered. Interestingly, high variability in the nucleotide sequence of the long terminal repeat (LTR) region that encodes the HIV-1 transcriptional promoter has been observed (Montano *et al.,* 1997, 1998; Verhoef *et al.,* 1999). Sequence analysis demonstrated a subtype-specific LTR-enhancer configuration with a low basal transcriptional activity that was strongly increased in the presence of Tat transactivator protein (Verhoef *et al.,* 1999). Nucleotide variations within the LTR promoter region of main subtypes B, C, and E include alterations in the TATA box, the NF-κB enhancer, the TAR element to which the Tat transactivator binds, as well as other mod-

[1] To whom correspondence and reprint requests should be addressed at Lady Davis Institute for Medical Research, McGill University, 3755 Cote Ste. Catherine, Montréal, Québec, Canada H3T1E2. Fax: (514) 340-7576. E-mail: jhisco@po-box.mcgill.ca.

0042-6822/02 $35.00

ulatory elements such as Sp1, USF, and NF-AT binding sites (Montano *et al.,* 1997, 1998; Jeeninga *et al.,* 2000). HIV-1 utilizes the NF-κB/Rel proteins to regulate transcription through NF-κB binding sites in the HIV-1 LTR (Kwon *et al.,* 1998). We have previously demonstrated that NF-κB is constitutively activated in primary monocytes and myeloid cell lines chronically infected with HIV-1 through the maintenance of a ternary NF-κB/DNA complex by IκBβ and virus-induced constitutive IKK activation (DeLuca *et al.,* 1999). NF-κB induces the expression of multiple cytokine, chemokine, growth factor, and immunoregulatory genes, many of which aid in HIV-1 replication (DeLuca *et al.,* 1999). Therefore, activation of NF-κB can impact upon HIV-1 replication and pathogenesis at many levels, highlighting the potential importance of altered κB sites in the various clade LTRs.

The HIV-1 Tat protein also exhibits amino acid divergence among the different clades, which may influence its binding and transactivation functions (Montano *et al.,* 1997, 1998). HIV-1 Tat stimulates transcription from the LTR promoter through an RNA hairpin element, called the transactivator protein-responsive region (TAR) (reviewed in Gatignol and Jeang, 2000; Karn, 1999). The function of HIV-1 Tat in resting $CD4^+$ T lymphocytes depends on κB-responsive elements within the LTR, again demonstrating the importance of sequence variations of NF-κB sites within the HIV-1 promoter (Hiscott *et al.,* 2001). The optimal activity of Tat is further dictated by its association with Tat-associated kinases (TAKs), which include the RNA polymerase II (RNAPII) C-terminal domain (CTD) kinase TFIIH and the cellular transcription elongation factor P-TEFb (Jones, 1997). TAKs affect processive transcription of RNAPII from the HIV-1 LTR promoter (Okamoto *et al.,* 1996; Parada and Roeder, 1996; Yang *et al.,* 1996). Recently, it has become apparent that the cofactor cyclin T1, a component of P-TEFb, forms a heterodimer with Tat and is recruited to the TAR (Garber *et al.,* 1998; Wei *et al.,* 1998). Once bound to the TAR, cyclin T1 and its associated proteins, particularly the CDK9 kinase, modify the RNAPII complex to form an elongation-competent state by phosphorylation of the C-terminal domain of RNAPII (Cullen, 1998). In addition, Tat-associated histone acetyltransferases, TAHs (which include p300 and p300/CBP-associating factor, PCAF), assist functionally in the activation of chromosomally integrated HIV-1 LTR, presumably through acetylation of histones (Benkirane *et al.,* 1998; Marzio *et al.,* 1998). Furthermore, p300 and PCAF also directly acetylate Tat on Lys50 in the TAR RNA binding domain and on Lys28 located in the activation domain, respectively (Kiernan *et al.,* 1999). In the present study, synergism between the histone deacetylase inhibitor trichostatin A and Tat was observed in transcriptional activation of the HIV-1 LTR. It was also observed that the κB sites of clade B and E LTRs bind NF-κB components; however, the duplicated κB sites of the C subtype do not compete for binding with NF-κB sites from HIV-1B or HIV-1E. Tat E, compared to Tat B or C, possesses the highest transactivation capacity, either alone or in combination with TNFα, regardless of the LTR context.

RESULTS

To analyze the regulatory interactions between the various HIV-1 LTR regions and the clade-specific Tat proteins, the LTRs of HIV-1B, HIV-1C, and HIV-1E were subcloned into the pGL3Basic vector. The selected HIV-1B, C, and E LTR and Tat sequences represent consensus sequences derived from analysis of more than 20 different strains from HIV^+ individuals for each clade (Montano *et al.,* 1997). The HIV-1 LTR promoter regions are schematically illustrated in Fig. 1A, indicating differences between the HIV-1B LTR versus clade C and clade E LTRs. Within the clade C LTR, duplication of the NF-κB sites is observed, resulting in two functional NF-κB sites and two putative κB sites. In contrast, within the HIV-1E LTR, one NF-κB site in the enhancer region has been replaced by a binding site for GABP, as previously described (Jeeninga *et al.,* 2000). As well, an additional TATA sequence has been found upstream of the NF-κB sites in the HIV-1 LTR E, but has been demonstrated to be nonfunctional (Jeeninga *et al.,* 2000). Other point mutations (illustrated as open circles) are located within other domains of the HIV-1 LTR. Figure 1B depicts the sequence comparison of the first 72 amino acids of the HIV-1 clade-specific Tat proteins. HIV-1B Tat is used as a reference strain since it represents the most widely studied Tat protein. Known acetylation sites occur at Lys28 and Lys50 within the Tat protein. Other amino acids representing putative acetylation sites are boxed. Additional sites are located at amino acids 24, 40, and 53 of Tat E and at amino acid 19 in Tat C. Figure 1C compares consensus HIV-1 Tat sequences (Con Tat B, Con Tat C, Con Tat E) for each of the clades (derived from the Los Alamos database) versus the HIV-1 Tat sequences used in this study. As Fig. 1 demonstrates, few alterations are observed within the selected Tat sequences, highlighting their value as representatives for each of their respective clades.

Binding of the NF-κB site within the clade B LTR was compared to the clade E LTR; specific oligonucleotide probes spanning the LTR region (PR-κB B, 5′ AGG GGA CTT TCC GCT GGG GAC TTT CCA G 3′; PR-κB E, 5′ CTA GGA CTT CCG CTG GGG ACT TTC CAG 3′) were used to examine the nuclear extracts derived from Jurkat cells treated or untreated with tumor necrosis factor α (TNFα) for the presence and inducibility of NF-κB. As observed in Fig. 2A, lane 2, an inducible protein DNA complex was observed in Jurkat cells treated with TNFα. Supershift analysis using specific NF-κB antibodies revealed that the complex was partially or completely supershifted by the addition of p50 and p60 subunit-specific antibodies (Fig. 2A, lanes 4 and 5). In addition, protein DNA com-

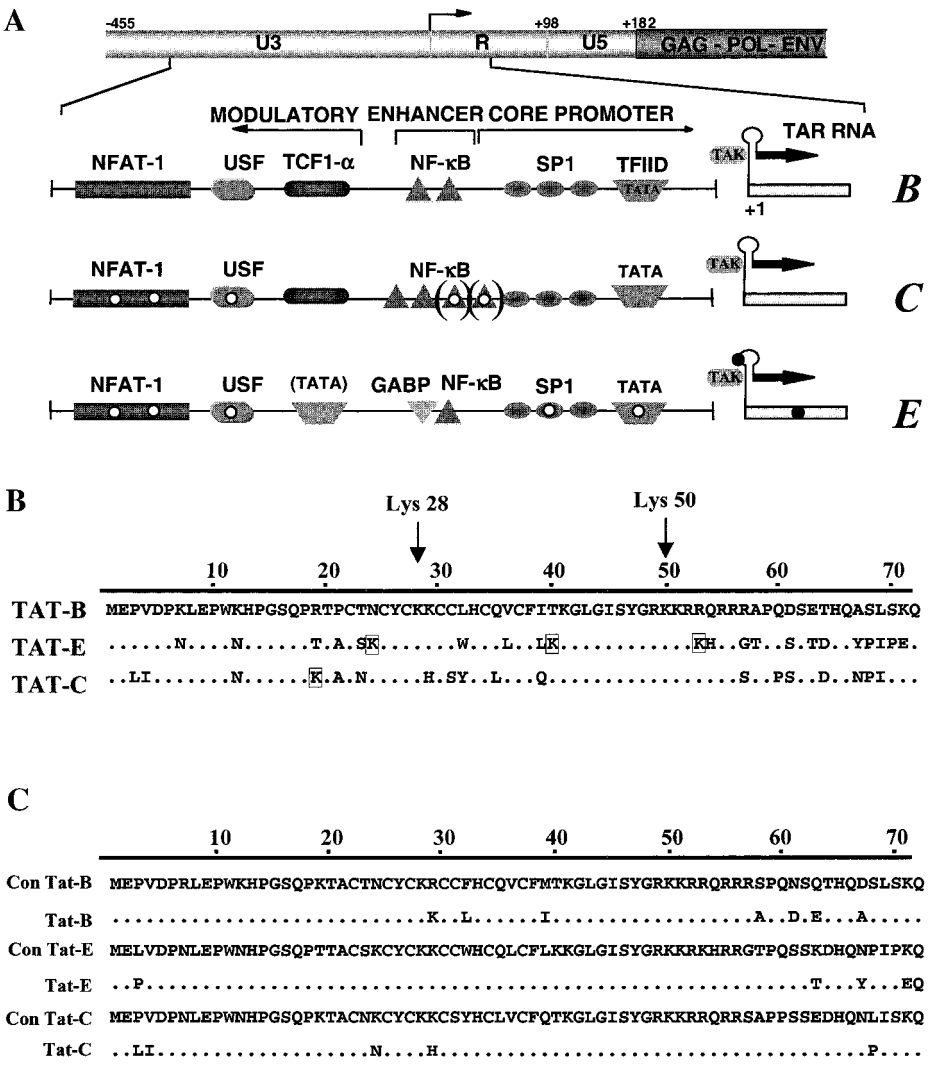

FIG. 1. (A) Comparison of LTR regulatory elements in HIV-1 clades B, C, and E. Enhancer and promoter elements included in the U3 and R regions of the LTR and reported to influence viral expression are shown schematically. Sequence variations within NFAT-1, USF, TCF1-α, Sp1, NF-κB, and the TATA box that distinguish the C and E subtypes from the B subtype are indicated by the open circles. The conversion of an NF-κB site into a GABP site within the E LTR subtype is shown as an inverted triangle (Verhoef *et al.,* 1999). Mutations in the clade E TAR element are indicated by closed circles. Also, the duplication of two putative NF-κB sites in the clade C LTR is shown in parentheses. (B) Sequence variation in subtype-specific Tat proteins. Amino acid residues (1–72 aa) in Tat E and C that differ from Tat B are shown. Lys28 and Lys50, which are targets of acetylation by PCAF and p300, respectively, are indicated by arrows. Additional lysine residues of Tat E and C that may be targets of acetylation are boxed. (C) Comparison of Tat B, C, and E sequences used in the present study versus known consensus sequences of each clade (derived from the Los Alamos database).

plexes containing a portion of c-Rel were supershifted by anti-c-Rel antiserum (Fig. 2A, lane 6). Using a κB-E probe, strong constitutive DNA binding activity was identified in Jurkat cell extracts. The intensity of the protein–DNA complex did not change with TNFα induction, indicating that the κB-E probe is modified such that it constitutively binds host cellular proteins. Using supershift analysis with p50, p65, and c-Rel, it was possible to demonstrate that the complex observed with κB-E contained p50, p65, and c-Rel (Fig. 2A, lanes 10–12); however, none of the antibodies produced a complete loss of the major complex. This binding may represent the strong interaction with GABP, as previously identified by Verhoef *et al.* (1999). In Fig. 2B, competition analysis was performed using different clade-specific oligonucleotides. Induction of U937 cells for 8 h with Sendai virus (SV) resulted in the appearance of a protein–DNA complex binding to clade B NF-κB sites (Fig. 2B, lane 2). Using either unstimulated or SV-induced extracts, competition experiments were performed using oligonucleotides corresponding to the NF-κB sites of the different HIV-1 LTR clades (B, C, or E clade). As shown in Fig. 2B, lanes 3 and 4, oligonucleotides B and E efficiently eliminated binding to the κB-B site. Similar results were obtained in extracts from SV-infected cells in which oligo(B) and oligo(E) competed for binding to the NF-κB site (Fig. 2B, lanes 6 and 7). However, in both instances HIV-1C oligonucleotide competitor (5′ CCT GGG GCG TTC CTG GGG CGT TCC AGG 3′) was unable to compete for the binding observed in U937 cells. Comparable results were obtained in TNFα-treated

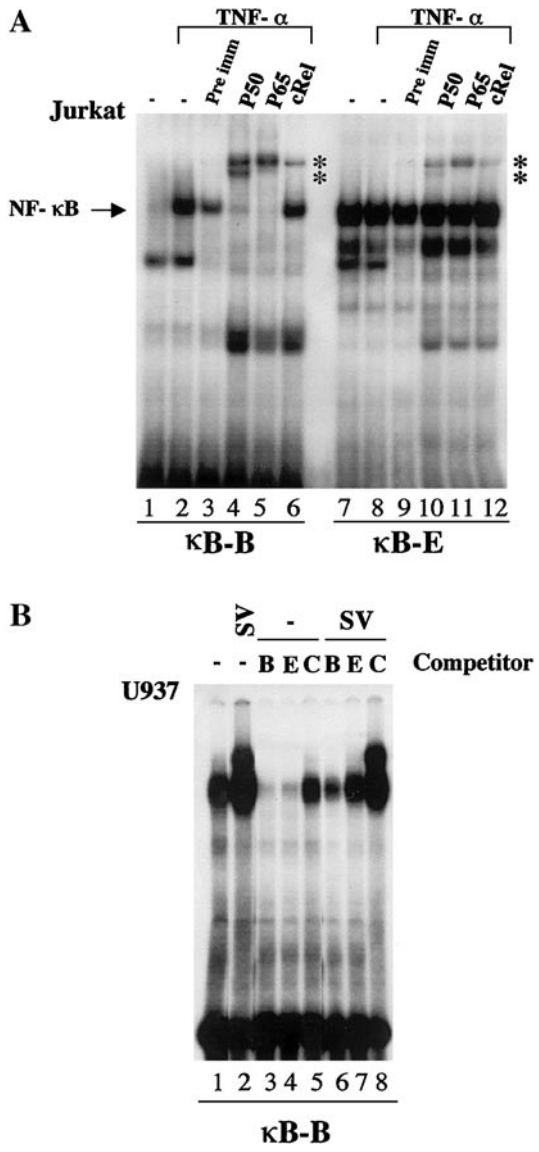

FIG. 2. EMSA analysis of the variant NF-κB sequences. (A) Jurkat T cells were stimulated with TNFα (10 ng/mL) for 1 h. Nuclear extracts (5 μg) were incubated with NF-κB sites from clade B (κB-B) and E (κB-E) LTRs (lanes 1–6 and lanes 7–12, respectively). The arrow indicates complex formation of p50, p65, and c-Rel, as determined by supershift analysis (lanes 4–6 and 10–12, respectively, as indicated by the asterisks). (B) U937 cells were infected (lanes 2, 6–8) with Sendai virus (80 HAU/ml) for 8 h; nuclear extracts (5 μg) were incubated with the κB-B probe and competition analysis was performed using oligonucleotides corresponding to the NF-κB sites of clade B, C, or E LTRs. Competition analyses were performed using a 50-fold excess of cold DNA oligonucleotides.

Jurkat cells (data not shown) and indicate that the duplicated sites of the clade C LTR are not *bona fide* κB elements. Together these results demonstrate that the κB-B and κB-E clade LTR elements are both capable of binding NF-κB subunits; however, NF-κB–clade B demonstrates inducible expression and binding of NF-κB subunits consisting of p50, p65, and to a much lesser extent, c-Rel. In comparison, the κB-E element shows strong constitutive binding and this binding activity could only be partially competed using antibodies against p50, p65, and c-Rel. Similarly, the competition analysis would suggest that the κB-B element has greater similarity with the κB-E element, given the ability of κB-E to compete for clade B DNA binding activity.

To analyze the effects of the different clade-specific Tat proteins on LTR activity, a series of cotransfection experiments was performed in Jurkat T cells and the results of the luciferase analysis of LTR activity are shown in Fig. 3. For each combination of LTR and Tat protein, the capacity of Tat in conjunction with TNFα to stimulate the LTR was evaluated. Based on the cumulative data of Fig. 3, several conclusions may be reached: the Tat E protein demonstrates the greatest fold activation, while Tat B shows the lowest, regardless of the LTR context; LTR C shows the least inducibility either with TCH alone or in combination with TNFα. However, it should be noted that the basal level of LTRC activity was approximately threefold higher than that of LTR B or LTR E, and thus Tat inducibility appears lower. In contrast to the expectation that homologous LTR–Tat combinations would preferentially transactivate, the results of this experiment indicate that the Tat E protein, regardless of the LTR context, is the strongest transactivator of LTR activity.

To investigate the possibility that deacetylation of Tat protein by histone deacetylases may influence Tat-mediated LTR activity, transactivation experiments were performed in the presence or absence of trichostatin A (TSA), a blocker of histone deacetylase activity. As seen

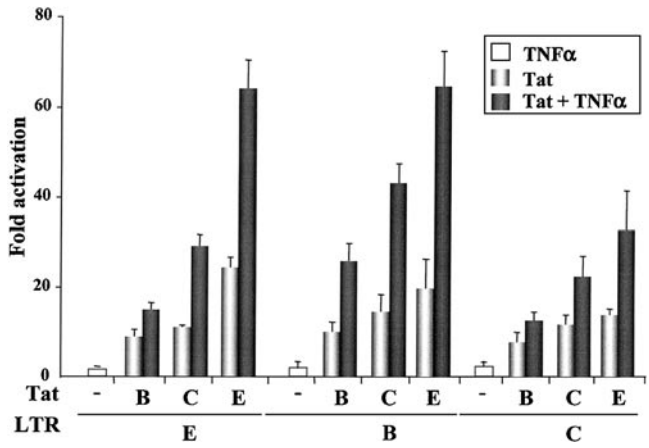

FIG. 3. Tat/TNFα synergistic transactivation of HIV-1 B, C, and E clade LTRs. Jurkat cells were transfected with luciferase promoter constructs under the control of E, B, or C clade LTRs in the absence (−) or in the presence of plasmids expressing the Tat protein from different clades (B, C, and E). Sixteen hours posttransfection, cells were incubated with 10 ng/ml of TNFα. After 10 h of stimulation, cells were assayed for luciferase activity. Relative luciferase activity (expressed as fold induction) is the ratio of luciferase activity between stimulated and unstimulated cells. Transfection efficiency was normalized to that of *Renilla* luciferase. Values are the mean of three independent experiments performed in duplicate. The FLU/RLU ratios for the unstimulated LTRs were 11.5 for E, 15.5 for B, and 33.8 for C.

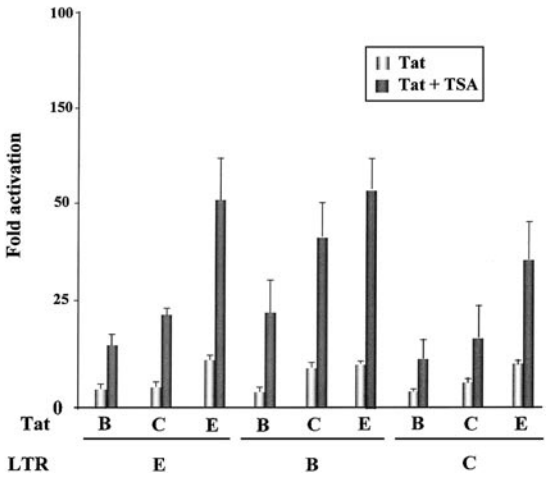

FIG. 4. Effect of TSA on Tat-mediated transactivation. Jurkat cells were transfected with luciferase promoter constructs under the control of E, B, or C clade LTR enhancer in the presence of plasmids expressing the Tat protein from different clades (B, C, and E). Sixteen hours posttransfection, cells were incubated with 450 nM TSA, a deacetylase inhibitor, or left untreated. After 10 h of stimulation, cells were assayed for luciferase activity. Relative luciferase activity (expressed as fold induction) is the ratio of luciferase activity between stimulated and unstimulated cells. Transfection efficiency was normalized to that of *Renilla* luciferase. Values are the mean of three independent experiments performed in duplicate.

in Fig. 4, the inhibition of deacetylation by TSA treatment led to a 3- to 4-fold increase for each clade-specific LTR, compared to LTRs analyzed in the presence of Tat alone. For example, the combination of LTR E/Tat E with TSA treatment produced a 4.4-fold increase compared to LTR E/Tat E in the absence of TSA treatment. In Fig. 5, induction of the different LTRs by Tat plus TNFα was analyzed in the presence or absence of TSA. Again, TSA treatment further stimulated Tat–TNFα transactivation of the LTR 3- to 4-fold depending on the Tat–LTR combination. For example, TSA treatment stimulated LTR E and Tat E transactivation by more than 3.5-fold. In all cases, the transactivator Tat E was the strongest activator of LTR activity, and its activity was augmented most dramatically by TSA.

DISCUSSION

Numerous studies have shown the existence of HIV-1 viruses with mosaic genomes (McCutchan *et al.,* 1999; Su *et al.,* 2000). To date, little is known about the effect of inter-subtype recombination of various HIV genomes in terms of the pathogenesis and replication efficiency of these viruses. Previous studies have shown that the LTRs from HIV-1 subtypes B, C, and E contain distinct sequence arrangements within the critical enhancer elements. HIV-1 subtype C isolates typically contain three functional NF-κB enhancer sites, while subtype B contains two (Montano *et al.,* 1998). These sites serve to upregulate viral transcription and replication in response to NF-κB/Rel family members in multiple cell types (Conant *et al.,* 1996; Granelli-Piperno *et al.,* 1995; Pahl, 1999; Hiscott *et al.,* 2001). Within the HIV-1E virus, a switch to a new binding site specificity from an NF-κB to a GABP site has been described, indicating the loss of a functional NF-κB site and the gain of a new specificity (Verhoef *et al.,* 1999). These genetic changes are characteristic for the respective subtypes and the NF-κB to GABP switch is present in all 18 subtype E sequences reported to date (Jeeninga *et al.,* 2000). It has been suggested that subtype E viruses are particularly virulent and replicate more proficiently than other subtypes in Langerhan cells, which are potential target cells in heterosexual transmission (Soto-Ramirez *et al.,* 1996). However, these data remain controversial (Pope *et al.,* 1997). Such molecular modification in the LTR may have profound consequences on viral replication, transmission, and dissemination.

In the present study, we observed differential basal activities of the LTRs of clade B, C, and E HIV-1 viruses as well as dissimilar transactivation potentials of the various Tat proteins. Transient transfection assays of Jurkat T cells indicate that Tat E, regardless of the LTR context, demonstrates the highest transactivation potential. This finding corroborates the results of Jeeninga *et al.* (1999), in which the replication efficiency of a wild-type (WT) clade B HIV-1 strain (LAI) was compared with a molecular clone of the strain in which the WT LTR B sequence was replaced with a portion of the LTR E sequence (Jeeninga *et al.,* 2000). The recombinant HIV-1B/E virus had an increased rate of replication compared to the WT clade B LAI strain (Jeeninga *et al.,* 2000), demonstrating that heterologous combinations of clade viruses may increase viral fitness.

Next, we sought to determine if acetylation of the

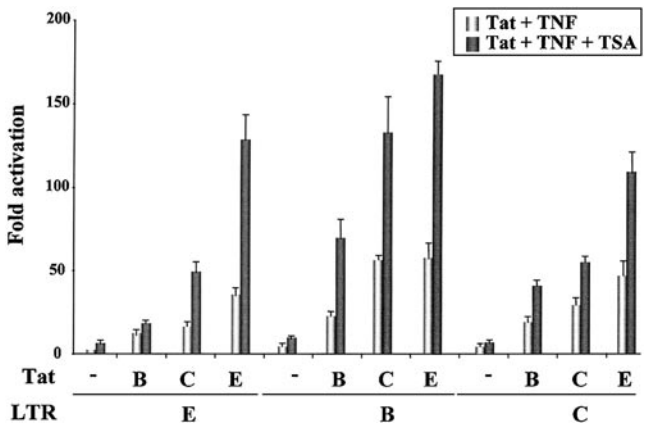

FIG. 5. Effect of TSA on Tat/TNFα synergism of LTR transcription. Jurkat cells were transfected with luciferase promoter constructs under the control of E, B, or C clade LTR enhancer in the presence of plasmids expressing the Tat protein from different clades (B, C, and E). Sixteen hours posttransfection, cells were incubated with 10 ng/ml of TNFα in the absence or in the presence of 450 nM TSA. Luciferase activity was determined as previously described in the legends to Figs. 3 and 4.

various Tat proteins plays a role in their transactivation capacity. It has previously been determined that acetylation of Tat is an important regulatory step in proper functioning of this transactivator. Acetylation of Lys28 by PCAF enhances Tat binding to CDK9/P-TEFb, whereas acetylation by p300 of Lys50 dissociates Tat from TAR, indicating a two-step time-dependent mechanism of Tat transactivational regulation by TAHs (Kiernan *et al.,* 1999). With addition of the deacetylase inhibitor trichostatin A, increased transactivation by each of the various Tat proteins was observed, with Tat E demonstrating the greatest fold induction. It is interesting to note that in addition to Lys28 and Lys50, Tat E contains further lysine residues at positions 24, 40, and 53 that may be subject to modification. However, it has been recently demonstrated that TSA plus TNFα, but not TSA alone, increased the expression of an NF-κB-dependent reporter gene through inhibition of histone deacetylase (HDAC) corepressor proteins that interact with the p65/RelA subunit of NF-κB (Chen *et al.,* 2001). Therefore the possibility exists that the greater transactivation seen in the presence of TSA may be dependent on the acetylation state of both Tat and NF-κB. Further analysis is required to determine if the various Tat proteins are diversely acetylated, i.e., if Tat E demonstrates a greater acetylation potential than Tat B or C, or if the effects of TSA are mediated mainly through NF-κB. An additional possibility to explain the high transactivation potential of Tat E is enhanced interaction with cyclin T1. Sequence variations between Tat proteins may account for the altered affinity with and binding of this cofactor, leading to the strong transactivation observed with Tat E. Currently studies are under way to determine if the differential transactivation of the clade-specific LTRs is due to Tat proteins or if the observed sequence variations within the LTR also play a major role, since LTR C was not activated well even in the presence of Tat E.

Variations between subclades of viruses may play an important role in their pathogenicity. Considerable evidence exists from animal retrovirus models for a role of LTR enhancer switches in regulating retrovirus-mediated pathogenicity and oncogenicity (Tsichlis and Lazo, 1991). Determining the importance of sequence variations among different clades of the HIV-1 may help to elucidate the biological consequences of such alterations and provide new avenues for therapy.

MATERIALS AND METHODS

Plasmid constructions

Tat sequences of HIV-1 clades B, C, and E were provided by Dr. Montano, Boston University, Boston, Massachusetts. The selected HIV-1B, C, and E LTR and Tat sequences represent consensus sequences derived from analysis of more than 20 different strains from HIV$^+$ individuals for each clade (Montano *et al.,* 1997). The selected clade-specific LTR and Tat represent sequences closest to consensus sequences found in the Los Alamos database. Briefly, Tat sequences of HIV-1 clades B, C, and E were subcloned into the pcDNA 3.1/Zeo (±) vector (Invitrogen) digested with *Hin*dIII and *Pst*I after the addition of adaptors to Tat sequences. pGL3/LTR B, pGL3/LTR C, and pGL3/LTR E constructs were created by digesting the 600-bp HIV-1 LTR clade B, C, or E from pβ-GalBasic (provided by Dr. Montano) with *Kpn*I and *Hin*dIII and were subcloned into pGL3Basic (Promega).

Cell culture, transfections, and luciferase assays

Jurkat T cells and U937 monocytic cells were cultured in RPMI medium (Gibco BRL) supplemented with 10% fetal bovine serum, glutamine, and antibiotics. Jurkat cells (10^6 cells per transfection), were cultured at 5×10^5 cells/ml for 16 h prior to transfection. HIV-1 LTR (clades B, C, and E) constructs (1 μg) and Tat (clades B, C, and E) constructs (1.2 μg) were transfected with 100 ng of pRL-tk (*Renilla* luciferase for internal control) using the FUGENE 6 transfection reagent (Roche) as indicated by the manufacturer. Sixteen hours posttransfection, cells were incubated with 10 ng/ml of TNFα. Cells were stimulated for 10 h with TNFα and luciferase activity was measured using the Dual Luciferase Reporter Assay System (Promega). For deacetylase inhibition assays, Jurkat cells were incubated with 450 nM trichostatin A and 10 ng/ml of TNFα 16 h posttransfection. After 10 h of stimulation, cells were assayed for luciferase activity.

Electrophoretic mobility shift assay (EMSA)

Jurkat cells were stimulated with TNFα (10 ng/ml) for 1 h, and U937 cells were incubated with Sendai virus (80 HAU/ml) for 8 h. The NF-κB DNA binding activity was determined by collection of nuclear extracts (10 μg) from Jurkat as well as U937 cells and EMSA was performed using ^{32}P-labeled probes, DNA binding buffer (10 mM HEPES, pH 7.9, 2% glycerol (v/v), 40 mM KCl, 1 mM EDTA, pH 8.0, 0.2 mM $MgCl_2$, 1 mM DTT, 0.05 mM PMSF), 0.2% NP-40, 0.5 μg of BSA, and 1 μg of poly(dI:dC). Incubation was performed for 30 min at room temperature using DNA oligonucleotides corresponding to the (−108 to −78) NF-κB region of the HIV-1 LTR: *PR-κB* B, 5′ AGG GGA CTT TCC GCT GGG GAC TTT CCA G 3′ (−108 to −80); *PR-κB C,* 5′ CCT GGG GCG TTC CTG GGG CGT TCC AGG 3′ (−108 to −81); *PR-κB E,* 5′ CTA GGA CTT CCG CTG GGG ACT TTC CAG 3′ (−105 to −78) (Montano *et al.,* 1997). NF-κB binding was confirmed using 1 μl of anti-p65, anti-p50, and anti-c-Rel antibodies (Santa Cruz Biotechnology Inc.). Competition analyses were performed using a 50-fold excess of cold DNA oligonucleotides.

ACKNOWLEDGMENTS

This research was supported by grants from the Canadian Institutes of Health Research and CANVAC, Network Centers of Excellence. P.R. was supported by a FRSQ–FCAR Studentship, M.R. by an NSERC Studentship, P.G. was supported by FRSQ Postdoctoral Fellowships, and M.A.W. and J.H. were supported by CIHR Senior Scientist awards.

REFERENCES

Benkirane, M., Chun, R. F., Xiao, H., Ogryzko, V. V., Howard, B. H., Nakatani, Y., and Jeang, K. T. (1998). Activation of integrated provirus requires histone acetyltransferase. p300 and P/CAF are coactivators for HIV-1 Tat. *J. Biol. Chem.* **273**(38), 24898–24905.

Carr, J. K., Salminen, M. O., Albert, J., Sanders-Buell, E., Gotte, D., Birx, D. L., and McCutchan, F. E. (1998). Full genome sequences of human immunodeficiency virus type 1 subtypes G and A/G intersubtype recombinants. *Virology* **247,** 22–31.

Chen, L., Fischle, W., Verdin, E., and Greene, W. C. (2001). Duration of nuclear NF-κB action regulation by reversible acetylation. *Science* **293,** 1653–1657.

Conant, K., Ma, M., Nath, A., and Major, E. O. (1996). Extracellular human immunodeficiency virus type 1 Tat protein is associated with an increase in both NF-kappa B binding and protein kinase C activity in primary human astrocytes. *J. Virol.* **70**(3), 1384–1389.

Cullen, B. R. (1998). HIV-1 auxiliary proteins: Making connections in a dying cell. *Cell* **93**(5), 685–692.

DeLuca, C., Kwon, H., Lin, R., Wainberg, M., and Hiscott, J. (1999). NF-kappaB activation and HIV-1 induced apoptosis. *Cytokine Growth Factor Rev.* **10**(3–4), 235–253.

Gao, M., Tsuchie, H., Jin, T. Q., Zhang, J., Detorio, M. A., Hossain, M. M., Taniguchi, K., and Kurimura, T. (1998). Difference in susceptibility to CC-chemokines among HIV-1 isolates. *Int. J. STD AIDS* **9**(8), 471–475.

Garber, M. E., Wei, P., Kewal Ramani, V. N., Mayall, T. P., Herrmann, C. H., Rice, A. P., Littman, D. R., and Jones, K. A. (1998). The interaction between HIV-1 Tat and human cyclin T1 requires zinc and a critical cysteine residue that is not conserved in the murine cycT1 protein. *Genes Dev.* **12**(22), 3512–3527.

Gatignol, A., and Jeang, K. T. (2000). Tat as a transcriptional activator and a potential therapeutic target for HIV-1. *Adv. Pharmacol.* **48,** 209–227.

Granelli-Piperno, A., Pope, M., Inaba, K., and Steinman, R. M. (1995). Coexpression of NF-kappa B/Rel and Sp1 transcription factors in human immunodeficiency virus 1-induced, dendritic cell-T-cell syncytia. *Proc. Natl. Acad. Sci. USA* **92**(24), 10944–10948.

Hiscott, J., Kwon, H., and Genin, P. (2001). Hostile takeovers: Viral appropriation of the NF-κB pathway. *J. Clin. Invest.* **107,** 143–151.

Jeeninga, R. E., Hoogenkamp, M., Armand-Ugon, M., de Baar, M., Verhoef, K., and Berkhout, B. (2000). Functional differences between the long terminal repeat transcriptional promoters of human immunodeficiency virus type 1 subtypes A through G. *J. Virol.* **74**(8), 3740–3751.

Jones, K. A. (1997). Taking a new TAK on tat transactivation. *Genes Dev.* **11**(20), 2593–2599.

Karn, J. (1999). Tackling Tat. *J. Mol. Biol.* **293**(2), 235–254.

Kiernan, R. E., Vanhulle, C., Schiltz, L., Adam, E., Xiao, H., Maudoux, F., Calomme, C., Burny, A., Nakatani, Y., Jeang, K. T., Benkirane, M., and Van Lint, C. (1999). HIV-1 tat transcriptional activity is regulated by acetylation. *EMBO J.* **18**(21), 6106–6118.

Korber, B. T., Allen, E. E., Farmer, A. D., and Myers, G. L. (1995). Heterogeneity of HIV-1 and HIV-2. *AIDS* **9**(Suppl. A), S5–S18.

Kwon, H., Pelletier, N., DeLuca, C., Genin, P., Cisternas, S., Lin, R., Wainberg, M. A., and Hiscott, J. (1998). Inducible expression of IkappaBalpha repressor mutants interferes with NF-kappaB activity and HIV-1 replication in Jurkat T cells. *J. Biol. Chem.* **273**(13), 7431–7440.

Marzio, G., Tyagi, M., Gutierrez, M. I., and Giacca, M. (1998). HIV-1 tat transactivator recruits p300 and CREB-binding protein histone acetyltransferases to the viral promoter. *Proc. Natl. Acad. Sci. USA* **95**(23), 13519–13524.

McCutchan, F. E., Carr, J. K., Bajani, M., Sanders-Buell, E., Harry, T. O., Stoeckli, T. C., Robbins, K. E., Gashau, W., Nasidi, A., Janssens, W., and Kalish, M. L. (1999). Subtype G and multiple forms of A/G intersubtype recombinant human immunodeficiency virus type 1 in Nigeria. *Virology* **254,** 226–234.

Montano, M. A., Nixon, C. P., and Essex, M. (1998). Dysregulation through the NF-kappaB enhancer and TATA box of the human immunodeficiency virus type 1 subtype E promoter. *J. Virol.* **72**(10), 8446–8452.

Montano, M. A., Novitsky, V. A., Blackard, J. T., Cho, N. L., Katzenstein, D. A., and Essex, M. (1997). Divergent transcriptional regulation among expanding human immunodeficiency virus type 1 subtypes. *J. Virol.* **71**(11), 8657–8665.

Okamoto, H., Sheline, C. T., Corden, J. L., Jones, K. A., and Peterlin, B. M. (1996). Trans-activation by human immunodeficiency virus Tat protein requires the C-terminal domain of RNA polymerase II. *Proc. Natl. Acad. Sci. USA* **93**(21), 11575–11579.

Pahl, H. L. (1999). Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* **18**(49), 6853–6866.

Parada, C. A., and Roeder, R. G. (1996). Enhanced processivity of RNA polymerase II triggered by Tat-induced phosphorylation of its carboxy-terminal domain. *Nature* **384**(6607), 375–378.

Pope, M., Frankel, S. S., Mascola, J. R., Trkola, A., Isdell, F., Birx, D. L., Burke, D. S., Ho, D. D., and Moore, J. P. (1997). Human immunodeficiency virus type 1 strains of subtypes B and E replicate in cutaneous dendritic cell–T-cell mixtures without displaying subtype-specific tropism. *J. Virol.* **71**(10), 8001–8007.

Robertson, D. L., Hahn, B. H., and Sharp, P. M. (1995). Recombination in AIDS viruses. *J. Mol. Evol.* **40**(3), 249–259.

Soto-Ramirez, L. E., Renjifo, B., McLane, M. F., Marlink, R., O'Hara, C., Sutthent, R., Wasi, C., Vithayasai, P., Vithayasai, V., Apichartpiyakul, C., Auewarakul, P., Pena Cruz, V., Chui, D. S., Osathanondh, R., Mayer, K., Lee, T. H., and Essex, M. (1996). HIV-1 Langerhans' cell tropism associated with heterosexual transmission of HIV. *Science* **271**(5253), 1291–1293.

Su, L., Graf, M., Zhang, Y., von Briesen, H., Xing, H., Kostler, J., Melzl, H., Wolf, H., Shao, Y., and Wagner, R. (2000). Characterization of a virtually full-length human immunodeficiency virus type 1 genome of a prevalent intersubtype (C/B′) recombinant strain in China. *J. Virol.* **74**(23), 11367–11376.

Takehisa, J., Zekeng, L., Ido, E., Yamaguchi-Kabata, Y., Mboudjeka, I., Harada, Y., Miura, T., Kaptu, L., and Hayami, M. (1999). Human immunodeficiency virus type 1 intergroup (M/O) recombination in Cameroon. *J. Virol.* **73**(8), 6810–6820.

Tsichlis, P. N., and Lazo, P. A. (1991). Virus–host interactions and the pathogenesis of murine and human oncogenic retroviruses. *Curr. Top. Microbiol. Immunol.* **171,** 95–171.

Verhoef, K., Sanders, R. W., Fontaine, V., Kitajima, S., and Berkhout, B. (1999). Evolution of the human immunodeficiency virus type 1 long terminal repeat promoter by conversion of an NF-kappaB enhancer element into a GABP binding site. *J. Virol.* **73**(2), 1331–1340.

Wei, P., Garber, M. E., Fang, S. M., Fischer, W. H., and Jones, K. A. (1998). A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. *Cell* **92**(4), 451–462.

Yang, X., Herrmann, C. H., and Rice, A. P. (1996). The human immunodeficiency virus Tat proteins specifically associate with TAK in vivo and require the carboxyl-terminal domain of RNA polymerase II for function. *J. Virol.* **70**(7), 4576–4584.

Zimmerman, P. A., Buckler-White, A., Alkhatib, G., Spalding, T., Kubofcik, J., Combadiere, C., Weissman, D., Cohen, O., Rubbert, A., Lam, G., Vaccarezza, M., Kennedy, P. E., Kumaraswami, V., Giorgi, J. V., Detels, R., Hunter, J., Chopek, M., Berger, E. A., Fauci, A. S., Nutman, T. B., and Murphy, P. M. (1997). Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5: Studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. *Mol. Med.* **3**(1), 23–36.