The HIV-1 Tat Protein Enhances Megakaryocytic Commitment of K562 Cells by Facilitating CREB Transcription Factor Coactivation by CBP

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The human immunodeficiency virus type 1 (HIV-1) Tat protein regulates transcription factor functions and alters cellular gene expression. Because hematopoietic progenitor cell (HPC) differentiation requires activation of lineage-specific transcription factors, Tat may affect hematopoiesis in HIV-1–infected microenvironments. We have monitored the molecular effects of Tat on megakaryocytic differentiation in the HPC line, K562. Flow cytometry analysis of CD61 indicated that phorbol myristate acetate (PMA) (16 n*M*) stimulated megakaryocytic commitment of K562 cells was increased (3- to 4-fold) following exposure to Tat (1–100 ng/ml). Activation of the megakaryocytic transcription factor cAMP regulatory element binding protein (CREB) and its coactivation by the CREB binding protein (CBP) was subsequently monitored. CREB phosphorylation and DNA binding were measured by Western immunodetection and electrophoretic mobility shift assays (EMSA), respectively. Within 2 hrs after stimulation, Tat increased both CREB phosphorylation and DNA binding by 7- to 10-fold. Transient cotransfection with CREB reporter and CBP expression plasmids demonstrated that Tat treatment increases (3- to 4-fold) both PMA-stimulated and CBP-mediated transcription via the cAMP regulatory element. Histone acetyl transferase (HAT) activity was increased (8- to 10-fold) in Tat-stimulated cells, which suggested increased chromosomal accessibility of transcription factors. Two-hybrid cotransfection assays using reporter plasmid containing the GAL4 DNA-binding domain and expression plasmid coding for the GAL4-CBP fusion protein, showed that Tat increases (2-fold) CBP-mediated coactivation of CREB. Both reverse transcription–polymerase chain reaction (RT-PCR) and Western blot analysis showed that Tat treatment increases CBP gene expression (7- to 9-fold) and protein levels (5- to 7-fold) within 6–12 hrs after stimulation. Our findings indicated that Tat treatment increases both CREB function and CREB coactivation by CBP, which may facilitate megakaryocytic commitment of K562 cells. Induction of this molecular signaling by HIV-1 Tat protein may have relevance in understanding the HIV-induced hematologic manifestations and possibly in regulation of viral infectivity parameters in progenitor cell reservoirs. Exp Biol Med 230:872–884, 2005

Key words: HIV-1; Tat; hematopoiesis; CREB; CBP; coactivation

Introduction

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of acquired immune deficiency syndrome (AIDS) and predominantly infects T-helper lymphocytes, monocytes, and macrophages (1, 2). Infection of lymphoid and myeloid lineages is mediated by recognition of the T-cell receptor CD4 or by the chemokine coreceptors CXCR4 and CCR5 (3–6). Although treatment with highly active antiretroviral therapy (HAART) is efficient in decreasing viral load in peripheral blood and delays the onset of AIDS (7–10), significant hematologic dysfunctions have been observed in patients infected with HIV-1, even those on long-term antiretroviral therapy. The hematologic abnormalities have been attributed to the deleterious actions of antiviral agents, especially the nucleoside reverse transcriptase inhibitors (NRTI) as well as to the virus itself within progenitor cell reservoirs of HIV-1 (11–14). The effects of HIV-1 propagation or exposure (or both) to different HIV-1–induced factors have also been linked to the dysfunctions in different hematopoietic progenitor cell (HPC) lineages (15, 16). Both *in vitro* and *in vivo* studies have shown a direct role for viral factors (e.g., gp120 and Tat) and virus-induced inflammatory mediators (e.g., tumor necrosis factor-α [TNF-α] and transforming growth factor-β1 [TGF-β1]) in altering the growth and differentiation potential of HPCs (17–22).

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However, the molecular mechanisms linked to the effects of each of these viral factors on lineage-specific differentiation of HPCs are not well understood.

Even though an early loss of circulating $CD34^+$ HPCs (22) and impaired clonogenic potential (23) and apoptosis of these progenitor cells (24) have been documented in individuals infected with HIV-1, the evidence of productive infection of HPCs remains controversial (25, 26). Although the peripheral blood–derived HPCs can express the HIV-1 coreceptors (27), susceptibility to either T-tropic or M-tropic strains of HIV-1 seem to correlate only with lineage commitment of HPCs, which leads to an increased expression of CD4, or to the CXCR4 and CCR5 coreceptors, or both (28). Productive infection by the X-4 and R-5 strains has been shown to occur only in HPCs committed toward the megakaryocytic and dendritic cell lineages (29–32). The absence of viral infection of HPCs makes it apparent that paracrine effectors secreted from HIV-1–infected cells may precipitate the hematologic dysfunctions observed in patients who test positive for HIV-1.

In our previously published studies we had documented that Tat exposure suppressed erythroid differentiation in erythroleukemic K562 cells (22). Recombinant Tat treatment as well as coculture with a Tat-expressing cell line (HL-Tat) significantly suppressed butyric acid–induced γ-globin gene expression and hemoglobin production in the K562 cells. Findings from our laboratory had suggested that HIV-1 (RF strain) can infect bone marrow cells and can constitutively express Tat protein (33). Both HIV-1 infection and exposure to Tat showed deleterious effects on mesenchymal stromal cell (MSC) growth and differentiation, and suppressed stromal hematopoietic support function. A number of reports have also shown that Tat can alter gene expression in different hematopoietic cell types (34–40).

In the milieu of deleterious agents produced within HIV-1–infected microenvironments, one of the key components is believed to be the HIV-1 Tat protein. Tat is known to be an essential regulatory protein for HIV-1 replication and is an early protein expressed at high levels even during the dormant stages of infection (41, 42). Tat accumulation in the nucleolus is responsible for the transcriptional activation of the provirus, which functions via recruitment of cellular factors to the HIV-1 long terminal repeat (LTR). Tat transactivation of the HIV-1 LTR requires a specific recognition of the trans-acting response (TAR) RNA element. In addition to this TAR-dependent effect, Tat protein can be secreted from infected cells and can have paracrine effects on neighboring cell types, which may be due to both extracellular and intracellular effects of Tat (43–45). The extracellular effects of Tat are believed to be due to the Asp-Gly-Arg (RGD) residues present within the second exon of Tat (amino acids 72–101). The first exon of Tat (amino acids 1–72), especially the lysine-rich core domain, has been implicated in manifesting the intracellular actions of Tat, which may regulate gene expression by altering the function of different transcription factors (46, 47). Multiple signal transduction intermediates have been shown to be affected by exposure of different cell types to the Tat protein (36, 44, 45). Although the effect of Tat on differentiation-specific signaling in HPCs has not been characterized, Tat may play a direct role in altering the function of lineage-specific transcription factors in HPCs. We hypothesized that the diverse effects of Tat protein may dysregulate lineage-specific differentiation in HPCs via the activation or inhibition (or both) of lineage-specific transcription factors and their interaction and sequestration via the coactivators.

The differentiation-specific signaling in HPCs brought about by humoral mediators and cytokines may be altered by viral factors that dysregulate the molecular actions of lineage-specific transcription factors and coactivators, thus altering differentiation-specific gene expression. It is interesting that in Jurkat T cells and in peripheral blood mononuclear cells (PBMCs), exogenously added Tat protein can activate ^{133}Ser-phosphorylation of cAMP regulatory binding protein (CREB) and increase CREB-dependent transcription (48). Recent findings have shown that the activation of transcription factor CREB and its interaction with the coactivator CREB-binding protein (CBP) are linked to the megakaryocytic lineage-specific differentiation of HPCs (49–51). The interaction of Tat with the transcriptional coactivators CBP and p300 has also been reported (51). These coactivators interact with the basal transcription apparatus in order for the transcription factors to activate gene expression and possess intrinsic histone acetyltransferase (HAT) activity, which is necessary to open the chromatin structure in order for gene expression to proceed (52–56). The coactivators CBP/p300 can associate with transcription factors activated via different signal transduction cascades and regulate their transcriptional ability. The paracrine effects of Tat protein in HIV-1–infected progenitor cell microenvironments may thus play a crucial role in dictating lineage-specific differentiation of HPCs.

In this study we provide *in vitro* evidence that exposure of K562 cells to the Tat protein can enhance their megakaryocytic commitment by activating the lineage-specific transcription factor CREB. At the molecular level, we observed that Tat activates CREB phosphorylation, DNA binding, and CREB-directed transcription. In addition, Tat was able to increase CBP expression and its coactivation function toward CREB-directed transcription.

Materials and Methods

Cell Culture and Treatment. K562 cells were obtained from American Type Culture Collection (Manassas, VA) and were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and penicillin-streptomycin (100 IU/ml-100 μg/ml) (Gibco-BRL, Gaithersburg, MD). Cells were passaged every 3 to 4 days at a 1:20 ratio. Cells were cryopreserved

($1–2 \times 10^6$ cells/ml) in medium containing 10% dimethylsulfoxide (DMSO) and freshly thawed cells were utilized after every 10–15 passages. For each experiment, confluent cultures were replenished with fresh medium and were cultured at a density of $2–3 \times 10^5$ cells/ml. In order to induce megakaryocytic differentiation, cells were stimulated with 16 n*M* phorbol myristate acetate (PMA) (Sigma, St. Louis, MO) alone or in the presence of 1–100 ng/ml of Tat (obtained from the National Institutes of Health AIDS Research and Reference Reagent Program, Rockville, MD). Cells were harvested within 60–90 mins after stimulation. Nuclear extracts were isolated to monitor HAT activity and CREB-specific oligonucleotide binding, and whole cell extracts were obtained to monitor CREB transcription factor phosphorylation status. For mitogen-activated protein (MAP) kinase inhibition studies, cells were incubated with 50 μ*M* PD-98059 (Sigma) for 15 mins before treatment with Tat, PMA, or both. For flow cytometric analysis of cell surface CD61, K562 cells were harvested at 24–36 hrs poststimulation with Tat, PMA, or both. In transient transfection experiments, cells were transfected with the CREB-reporter plasmid alone or in conjunction with the Tat or CBP expression plasmids (or both). Cells were stimulated with PMA, or Tat, or both at 16 hrs after transfection and were harvested at 24 hrs after stimulation. In two-hybrid assays, cells were transfected with the CBP-GAL4 fusion plasmid and the GAL4-Luc reporter plasmid to monitor CBP function, and similar transfection and treatment protocols were carried out.

Nuclear and Whole Cell Protein Extraction. The nuclear and whole cell extracts from control and treated K562 cells were obtained by using the M-per detergent-based cell lysis assay (Pierce Bio-Tech, Rockford, IL). Lysates were stored at –80°C for later use in immunodetection analysis of CREB phosphorylation and CBP protein levels. For electrophoretic mobility shift assays (EMSAs), nuclear protein extracts were obtained using a nuclear protein extraction kit (Promega, Madison, WI). Samples were centrifuged at 20,000 *g* for 5 mins at 4°C, and supernatants were snap-frozen in liquid nitrogen and stored at –80°C for later use.

Western Immunodetection. Whole cell extracts (25–35 μg protein) were used in these assays. Samples were thawed and 5 μl of gel loading buffer containing 1% sodium dodecyl sulfate (SDS) and 1 μl of β-mercaptoethanol were added. Samples were boiled for 3 mins and electrophoresed in tris-glycine/SDS buffer at 90 amps for 4 hrs using 4%–29% gradient polyacrylamide gel (Bio-Rad, Hercules, CA). Electrophoresed samples were transferred to nitrocellulose using a semidry transfer apparatus (Bio-Rad). The nitrocellulose filters (Pierce Bio-Tech) were incubated in Tris-buffered saline containing 0.05% Tween (TBST) and 5% nonfat dry milk (Sigma) for 1 hr to block nonspecific binding. Polyclonal human anti-CREB antibody or antibody directed against the CREB oligopeptide phosphorylated at the serine moiety at position 133 (CREB-PSer^{133}), as well as antibodies to human CBP were obtained from Upstate Biotechnology Inc. (Lake Placid, NY). Cell extracts were also monitored for total β-actin levels using human antiactin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) and were used as internal controls. The primary antibodies were diluted (1:1000) in TBST buffer and blots were incubated overnight at 4°C, followed by four washings in TBST for 10 mins and then incubated in a 1:2000 dilution of goat anti-rabbit horseradish peroxidase-conjugated secondary antibody for 1 hr. Blots were exposed to the Super-signal chemiluminescent substrate (Pierce Diagnostics, Rockford, IL) for 5 mins and exposed to x-ray films. Analyses of band intensities were carried out using a GS-700 Densitometer (Bio-Rad).

Electrophoretic Mobility Shift Assay. The CREB-recognition element (CRE) consensus oligonucleotides (5′-AGA GAT TGC **CTG ACG TCA** GAG AGC TAG-3′ and 5′-CTA GCT TCT TGA CGT CAG GCA ATC TCT-3′) were purchased from Santa Cruz Biotechnology. The oligonucleotides were end-labeled using high specific activity (7000 Ci/mmol) (γ-^{32}P) ATP (Amersham Pharmacia Biotech, Piscataway, NJ), and T4-polynucleotide kinase (Promega). Nuclear protein–DNA binding reactions were carried out at room temperature for 20 mins in a volume of 20 μl containing 20 μg of nuclear protein, 10 m*M* Tris–HCl (pH 7.5), 50 m*M* NaCl, 1 m*M* EDTA, 5% glycerol, 1 m*M* dithiothreitol, and 1 μg of poly(dI-dC) as a nonspecific competitor. The DNA-protein complexes were resolved by electrophoresis on 6% polyacrylamide gels containing 50 m*M* Tris, 0.38 *M* glycine, and 2 m*M* EDTA. The gels were dried and autoradiographed following exposure of the x-ray films at empirically determined time intervals.

Plasmid Constructs. The CREB reporter plasmid (pCREB3/ho1-CAT) containing three tandem CRE elements inserted upstream of the heme-oxygenase-1 (HO-1) promoter, which regulates transcription of chloramphenicol acetyltransferase (CAT) gene, was kindly provided by Dr. J. Alam (57). The CBP expression plasmid under the control of cytomegalovirus (CMV) promoter (pCMV-CBP) was kindly provided by Drs. S.S. El-Dahr and R. Kwok (58). The TF-IID promoter-driven CBP and GAL-4 chimera expression plasmid (pCBP-GAL4) and the Gal4-responsive luciferase reporter (pGAL4-Luc) were kindly provided by Dr. M.E. Burow (59). For large-scale cloning purposes, the plasmids were transformed into competent *Escherichia coli* (strain JM-109), and a Qiagen (Valencia, CA) plasmid maxi kit was used to isolate and purify each of the plasmids. Plasmid concentrations were measured by spectrophotometric analysis at 260 nm wavelength, and aliquots (1 μg/μl) were frozen at –20°C.

Transient Transfection Assays. The CREB-directed transcription assays were carried out with lipofectamine-plus (Invitrogen, Carlsbad, CA) and the two-hybrid assays were carried out using the effectene reagent (Qiagen). Both transfections were carried out in accordance with the

manufacturer's protocols. In experiments using lipofectamine-plus, K562 cells (5×10^6) were transfected with approximately 10.0 μg of the reporter construct (pCREB3/hol-CAT). In specified experiments, 1.0 μg of the CBP expression plasmid (pCMV-CBP) was also cotransfected into cells. In each experiment, the transfection efficiencies were monitored using a β-galactosidase expression plasmid (pCMV-βGal) and monitoring of β-galactosidase activity in cell extracts. Approximately 16–20 hrs post-transfection, cells were incubated with recombinant Tat protein (1–100 ng/ml) or PMA (16 n*M*), and treatment was carried out for another 24 hrs. Cell extracts were obtained by using Triton-X lysis buffer. For the CAT assays, extracts containing 60–100 μg of protein were incubated in CAT assay buffer containing 5 μCi of (^{3}H)acetate (ICN, Cleveland, OH), 20 m*M* ATP, 50 m*M* coenzyme A, 2.5 m*M* chloramphenicol, and 0.05 U of S-acetyl coenzyme-A synthetase. Incubation at 37°C was carried out for 2–4 hrs, after which chloramphenicol acetate was isolated by extraction with benzene. The (acetyl-^{3}H) chloramphenicol was extracted from the organic phase and was counted using a scintillation counter. The protein content in supernatant was determined by using the Bio-Rad assay kit, and the CAT activities were normalized for each sample. For the two-hybrid assays, cells (8×10^5 cells/ml) were transfected with 200 ng of pCBP-GAL4 and 100 ng of either pGal4-Luc or the pEG2 control vector. Approximately 16–20 hrs after transfection cells were centrifuged and resuspended in the presence or absence of Tat (1–100 ng/ml) or PMA (16 nM) for 24 hrs. The cell extracts were obtained using the reporter lysis buffer, and extracts were normalized to total protein content in each sample. To ascertain luciferase expression, we used a single luciferase substrate in conjunction with a tube luminometer set to inject 100 μl of luciferase substrate into tubes containing 20 μl of sample. Gal-4 responsive luciferase activity was correlated with an increase in CBP mediated transcriptional activity.

HAT Activity Assays. HAT activity was detected using the Hat-Check system from Pierce Biotechnology. In this system, the acetylation of a protein fragment corresponding to the first 23 residues of histone-4 (H-4) conjugated to biotin was monitored and experiments were carried out according to the manufacturer's instructions. Briefly, equal amounts of nuclear extracts (60–100 μg protein) were diluted in a reaction mixture containing the histone peptide–biotin conjugate, HAT-Check reaction buffer, and ^{3}H-acetylcoenzyme-A, and were incubated at 37°C for 1 hr. The samples were incubated with streptavidin-agarose beads for 30 mins, after which the slurry was washed three times with radio-immuno-precipitation buffer, resuspended in 500 μl of HAT-buffer, and bead-associated radioactivity was monitored by using a liquid scintillation counter.

RNA Isolation and Reverse Transcriptase–Polymerase Chain Reaction Assays. Isolation of total cellular RNA was carried out by using a Tri-Zol kit (Promega). The concentration of the total RNA was determined, and purity and integrity of the RNA was verified by electrophoresis in 1.2% agarose gel containing formaldehyde and aliquots were stored at –70°C for later use in reverse transcriptase–polymerase chain reaction (RT-PCR) studies in freshly thawed RNA samples. Briefly, 0.5 μg of total RNA was incubated with avian myeloblastosis virus reverse transcriptase (0.5 U) (Promega) for 15 mins at 42°C. Equal amounts of the cDNAs were used in PCR amplification of CBP (60) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (38) using RED-Taq DNA polymerase (0.5 U) and dATP, dTTP, dCTP, and dGTP (10 m*M* each) (Promega) according to previous published studies (38, 60). The PCR products were electrophoresed on a 2.0% agarose gel and the band intensities were densitometrically quantified using a GS-700 Densitometer (Bio-Rad). The RT-PCR primers were synthesized from Midland Certified Reagent Company (Midland, TX) and the sequences were as follows:

CBP: 5′-AGTGGA ATTCAAACCACAATTGGTTC-TGTTGGTGCAGGGCA-3′ and
5′-TAAAGCTGGCTGGTTACCCAGGATGC-CTTGCTTATGTAAACG-3′.

GAPDH: 5′-GAAGTTGAAGTTCGGAGTCAACG-3′ and
5′-TGCCATGGGTGGAATCATATTGG-3′.

Flow Cytometry Analysis. Cells ($1–2 \times 10^6$ cells/ml) were labeled with 20 μl of fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibody directed against human CD61, clone VIPL2 (BD Biosciences, Bedford, MA). Following 20 mins of incubation, cells were washed with PBS and resuspended in PBS containing 2% formaldehyde for 1 hr. Flow cytometry was performed using the fixed cells within 24 hrs. Cells were gated using unstained cells and nonspecific binding was monitored using mouse antibodies to appropriate isotypes (BD Biosciences Pharmingen; mouse IgG). In each experiment, FITC-specific fluorescence (FL1) was measured in approximately 10,000 cells in duplicates by using a BD-Coulter Epic-xl flow cytometer. Both the percentage change in CD61-expressing cells and the increase in CD61-specific mean fluorescence intensity (MFI) were monitored in each experiment.

Statistical Analysis. All statistical analyses were performed with Instat-2 software (GraphPad, San Diego, CA). Experiments were performed at least three to five times, and the values obtained from two to four replicate samples were averaged in each experiment. The results are expressed as standard errors of mean (± SEM). The significance of changes from control values was determined by using a two-tailed Student's *t* test and *P* values of < 0.05 were considered to be significant.

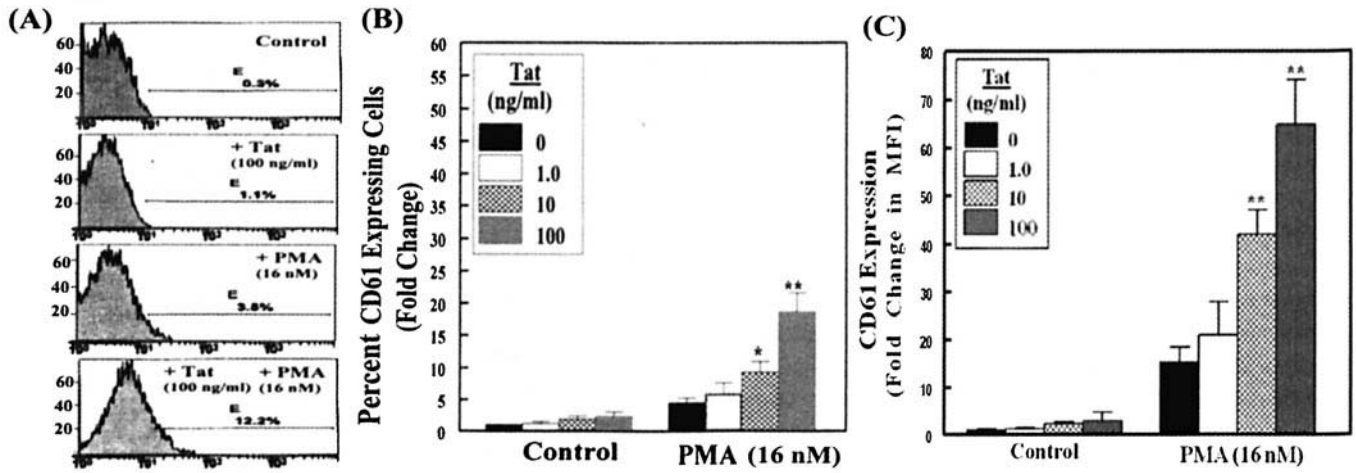

Figure 1. Effect of HIV-1 Tat protein on CD61 expression in PMA-stimulated K562 cells. Cells were exposed to PMA alone or in combination with recombinant Tat protein for 24 hrs. Flow cytometric analysis was performed to monitor the expression of CD61. Unstained cells were used to set the gating and CD61-positive cells were monitored in region 'E'. In (A), a representative flow cytometry data showing the effect of Tat (100 ng/ml) alone and in combination with PMA (16 n*M*) cotreatment are shown. In (B), data obtained are depicted as a fold change in the percentage of CD61-expressing cells. In (C), data obtained are depicted as a fold change in CD61-specific mean fluorescence intensity (MFI). Bar graphs depict fold differences from five independent experiments ($n = 5$) and error bars represent the ± SEM of values. Significant changes following Tat, or PMA stimulation (or both), compared with unstimulated controls (*$P < 0.05$ and **$P < 0.01$).

Results

Tat Cotreatment Enhances PMA-Stimulated CD61 Expression in K562 Cells. PMA-induced megakaryocytic differentiation of K562 cells was monitored by flow cytometric analysis of CD61 expression (Fig. 1). Cell surface CD61 levels were monitored using an FITC-conjugated anti-human CD61 antibody. The unstained cells were used to monitor background fluorescence and positively stained cells were gated into region 'E'. Representative flow cytometry data obtained with the highest concentration of Tat (100 ng/ml) and coexposure to PMA (16 n*M*) are shown in Figure 1A. Average fold changes in CD61 expression in response to increased concentrations Tat (1–100 ng/ml) in the presence or absence of PMA (16 n*M*) are shown as a percentage change in cell numbers (Fig. 1B) as well as a fold change in MFI (Fig. 1C). Compared with the unstimulated controls, an increase in CD61-expressing cells was observed in cells stimulated with 16 n*M* PMA. Tat treatment alone did not show a significant change at 1.0 or 10.0 ng/ml, although at 100 ng/ml of Tat an increase of approximately 2-fold was observed in CD61-expressing cells. However, Tat costimulation was able to significantly ($P < 0.05$) enhance (by 2- to 3-fold) the PMA-induced percentage of CD61-expressing cells (Fig. 1B) and increase (3- to 5-fold) in CD61-specific MFI (Fig. 1C). The enhancement in PMA-induced CD61 levels were observed consistently at all concentrations of Tat tested; however, the significant increase was evident at 10 and 100 ng/ml of Tat coexposure. Thus, Tat coexposure enhanced megakaryocytic commitment and increased CD61 expression in PMA-stimulated K562 cells.

Rapid Increase in ^{133}Ser-CREB Phosphorylation in Tat-Stimulated Cells. Activation of CREB (p38) transcription factor is an early determinant of megakaryocytic commitment of $CD34^+$ HPCs, which is dependent on the activation of MAP-kinase signal transduction cascades (49). We monitored the effect of Tat on CREB phosphorylation in K562 cell extracts by using both nonphosphorylated and phopho-specific CREB antibodies in Western immunodetection analysis. Results obtained from K562 cells stimulated with either 10 ng/ml or 100 ng/ml of Tat protein are shown in Figure 2A, with the bands obtained following incubation with antibodies toward either the phosphorylated (upper bands) or the nonphosphorylated (lower bands) CREB. Densitometric analysis of the phosphorylated CREB bands was normalized to that obtained with the nonphosphorylated CREB (total) contents in each sample. The ratio obtained in unstimulated control cultures was empirically designated as 1.0. The fold changes in ^{133}Ser-P CREB levels following Tat treatment are shown at the bottom of the gel pictures. As seen in Figure 2A, Tat stimulation increased ^{133}Ser-P CREB levels within 60 mins, whereas no detectable change was evident with the nonphosphorylated constitutive CREB levels. Furthermore, this increase was concentration-dependent on the amount of Tat protein used, which showed a 3-fold and a 7-fold induction in ^{133}Ser-P CREB levels with 10 ng/ml and 100 ng/ml of Tat, respectively. Preincubation of K562 cells for 15 mins with PD-98059 (50 μ*M*) completely abolished Tat-induced ^{133}Ser-P CREB levels, suggesting a role for the MAP kinase signaling cascade in Tat-mediated CREB phosphorylation. These results indicate that Tat exposure facilitates the early commitment step in megakaryocytic differentiation of K562 cells.

Tat Treatment Increases CREB Binding to the CRE Element. In the following studies, we investigated whether Tat-mediated activation of CREB may have a direct role in the functional enhancement of CREB binding to the

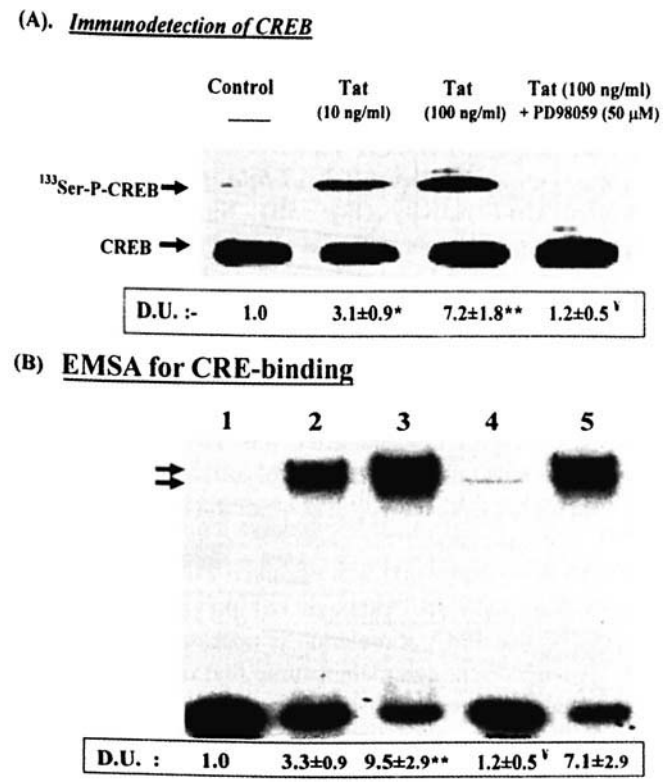

Figure 2. Effect of Tat on CREB phosphorylation and DNA binding. K562 cell nuclear extracts were monitored for CREB activation and CRE binding activity. In (A), cells were treated with Tat (10 and 100 ng/ml) for 90 mins in the presence or absence of the MAP kinase inhibitor PD98059 (50 μM). Western immunodetection was carried out using antibodies specific for both the phosphorylated and nonphosphorylated forms of CREB. In (B), EMSAs were carried out using nuclear extracts from control (Lane 2) and Tat (100 ng/ml)-stimulated cells (Lanes 3–5). The ^{32}P-labeled CRE consensus oligonucleotides alone (Lane 1) or following incubation with nuclear extracts from Tat-treated cells (Lanes 2–5) are shown. Specific binding was assessed by incubation in the presence of excess (100×) unlabeled oligonucletides (Lane 4) or with noncompetitor oligonucleotides (Lane 5). In both (A) and (B), band intensities were quantified densitometrically. In (A), values obtained for phosphorylated CREB were normalized to the respective values obtained for nonphosphorylated CREB bands. Fold changes in empirical densitometric units (D.U.) obtained from three independent experiments (n = 3) are shown at the bottom of each gel along with the ± SEM of values. Significant changes in ^{133}Ser-P CREB in Tat-treated cells are shown as P values (*$P < 0.05$ and **$P < 0.01$) and ¥ for $P < 0.01$ in PD98059-exposed cells. In (B), significant changes in EMSA in Tat-treated nuclear extracts (**$P < 0.01$) and following competition with excess unlabeled oligonucleotides (¥$P < 0.01$) are shown.

CREB recognition element (CRE). Nuclear extracts from control and Tat (100 ng/ml)-stimulated cells were monitored for protein binding to the consensus CRE oligonucleotides by using EMSAs (Fig. 2B). The CRE oligonucleotides were radiolabeled, incubated with K562 nuclear extracts, electrophoresed on a polyacrylamide gel, and the intensities of shifted bands were monitored. Migration of the unbound oligonucleotide probe is shown in Figure 2B, Lane 1. Protein binding in unstimulated (Lane 2) and Tat-stimulated (Lane 3) nuclear extracts demonstrated that Tat treatment can increase protein binding to the CRE oligonucleotides within 90 mins after stimulation. Densitometric analysis of the shifted bands revealed a 4- to 5-fold increase in CRE binding compared with that observed in the unstimulated control cells. This increase in transcription factor binding was specific and could be competed out by incubation with 100-fold excess of unlabeled CRE oligonucleotides (Lane 4), but not with the random oligonucleotides (Lane 5). These observations on increased CRE binding in conjunction with the increased CREB phosphorylation data suggested that Tat activates CREB-mediated transcription in K562 cells. These data also suggested that Tat treatment alone may directly enhance the commitment of K562 cells toward megakaryocytic differentiation even in the absence of PMA stimulation. However, these findings were not able to elucidate why PMA stimulation was required for the Tat-induced increase in CD61 expression in K562 cells. Tat-mediated effects on CREB phosphorylation and DNA binding may not be sufficient for CREB-directed gene expression.

CREB-Directed Gene Expression in PMA-Stimulated Cells Is Enhanced Following Tat Cotreatment. In order to investigate whether Tat treatment may increase CRE-directed gene expression, K562 cells were transiently transfected with a CREB reporter plasmid (pCREB3/hol-CAT). Approximately 16–20 hrs after transfection, cells were stimulated with Tat (1–100 ng/ml) alone or in conjunction with PMA (16 nM) for 24 hrs, and CAT activity was monitored in cell extracts (Fig. 3A). The bar graphs in Figure 3A show the average CAT activity (cpm × 10^{-2}) obtained in three separate transient transfection experiments (n = 3). Compared with the unstimulated controls, stimulation with the lower concentration of Tat (1 ng/ml) did not increase CREB-directed gene expression; however, exposure to 10 or 100 ng/ml of Tat increased CAT activity by 1.5- to 2.2-fold (hatched bars). Stimulation with PMA showed at least a 2-fold increase in CAT activity (solid bars), and Tat cotreatment showed a further increase in CREB-directed CAT gene expression. Cotreatment with PMA and Tat (1 ng/ml) showed a detectable increase in CREB-dependent transcription, and significantly increased CREB activity was observed in cells stimulated with 10 or 100 ng/ml of Tat, which showed an almost 3-fold enhancement of PMA-induced effect. These results showed that Tat alone could activate CREB-directed transcription, similar to the data obtained with CREB phosphorylation and DNA binding (Fig. 2), however, it also indicated that PMA stimulation was necessary for manifesting significant increases in CREB-directed transcriptional effects of Tat, which was similar to the data obtained for CD61 expression studies (Fig. 1).

CBP Overexpression Circumvents the Need for PMA During Tat-Induced CREB Function. The findings described above implicate the requirement for parallel signaling pathways that may be activated via PMA, and are required for a Tat-mediated increase in CREB-directed transcription. CREB requires CBP to activate transcription (53, 54, 61), and a number of studies have shown that the

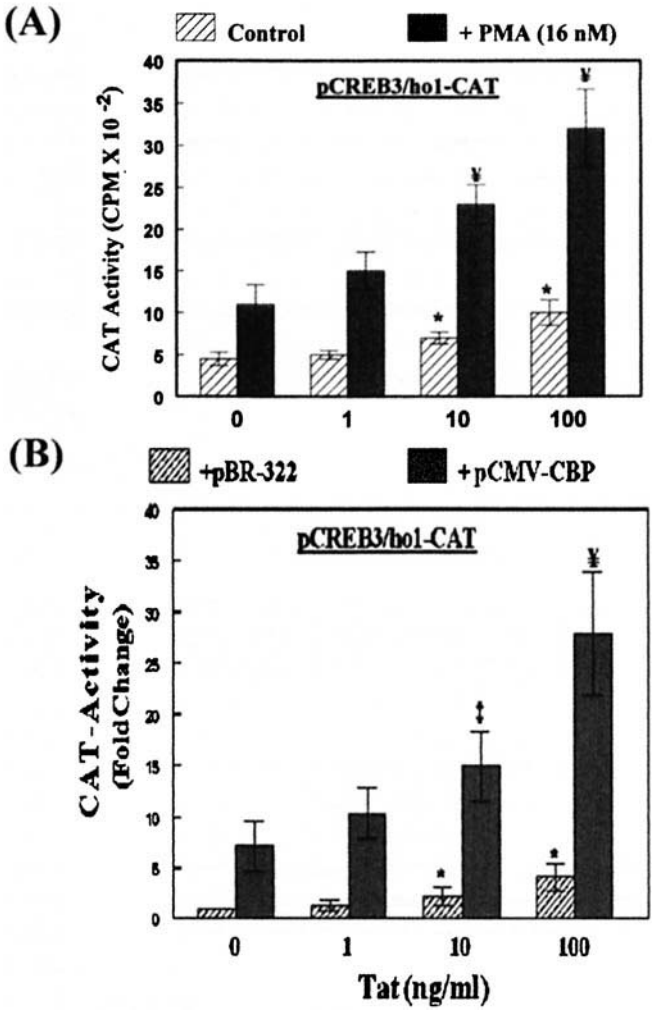

Figure 3. Effect of Tat on CRE-directed transcription in the presence or absence of CBP overexpression. CRE-directed gene expression was monitored in K562 cells transiently transfected with pCREB3/ho1-CAT. (A) Cells were treated with Tat (1–100 ng/ml) alone or in combination with PMA (16 n*M*) and CAT activity was monitored at 24 hrs after treatment. The bar graphs represent average counts per minute (cpm $\times$ 10^{-2}) obtained from three separate transfection experiments ($n = 3$) and error bars represent $\pm$ SEM of values. Significant changes from unstimulated controls are represented as *P* values ($^*P < 0.05$ and $¥P < 0.02$). (B) Effect of Tat on CREB-directed transcription in CBP overexpressing cells. Cells were transfected with pCREB3/ho1-CAT, and cotransfected with either the empty vector (pBR322) or the CBP expression vector (pCMV-CBP). Cells were treated with Tat (1–100 ng/ml) at 16 hrs after transfection and harvested at 24 hrs to monitor CAT activity. Average fold change obtained from three independent experiments ($n = 3$) are represented in the bar graphs, and error bars represent the $\pm$ SEM of values. Significant change in CREB-directed CAT enzyme activity in the presence of CBP overexpression is represented as $^*P < 0.02$, and significance of changes following Tat costimulation are represented as $‡P < 0.05$ and $¥P < 0.01$.

Tat protein can also interact with CBP and alter its coactivator function (51, 62, 63). Hence, CREB association with the transcriptional coactivator CBP was assessed in the following experiments. We investigated whether the requirement for PMA stimulation to induce Tat-mediated effects on CREB-directed transcription can be overcome by CBP overexpression. The K562 cells were transfected with pCREB3/ho1-CAT alone or cotransfected with the CBP expression plasmid, pCMV-CBP. To determine the background levels of CRE-directed gene expression, control cells were cotransfected with similar quantities of pBR322 and the pCREB3/ho1-CAT vector. Overexpression of CBP resulted in an approximately 7-fold increase in CRE-directed CAT activity (Fig. 3B). Similar to previous experiments, treatment with Tat alone was able to increase CREB-directed CAT transcription (2- to 3-fold). In addition, in CBP overexpressing cells, exogenous addition of Tat was able to significantly increase CAT activity. However, we observed that only the 10 ng/ml or 100 ng/ml concentrations of Tat were able to increase CREB-directed transcription, but the 1 ng/ml concentration was not able to do so. Compared with unstimulated control cells, as much as 28- to 35-fold higher CAT activity was observed in the presence of CBP overexpression. This phenomenal increase in CREB-directed gene expression was observed even in the absence of PMA stimulation. Although our previous experiments suggested that PMA stimulation is necessary for the effect of Tat, the current data demonstrate that overexpression of CBP can circumvent the need for PMA, possibly via an enhancement in CREB-CBP interactions due to the increased availability of the transcriptional coactivator.

Tat Treatment Increases HAT Activity in K562 Cell Extracts. Tat-mediated activation of CREB may be due to an increased association with CBP, which may result in an increase in CBP-mediated HAT activity. Transcriptional activation is linked to an increase in nuclear HAT activity, which leads to enhanced chromosomal accessibility of transcription factors (54, 56). Hence, in order to determine whether Tat can increase CBP function we first investigated whether Tat can enhance HAT activity in K562 cells (Fig. 4A). Nuclear extracts were obtained from cells stimulated with increasing concentrations of Tat (0.1–100 ng/ml) for 2 hrs, and HAT activity was monitored. The data indicated that Tat treatment significantly increased HAT activity as measured by an increase in HAT-associated radioactivity (cpm). A 2- to 3-fold increase in HAT activity was evident even with 0.1 ng/ml of Tat, and a 6-fold increase was observed with 1.0 ng/ml of Tat. A slightly higher HAT activity was observed with the higher concentrations of Tat (10 or 100 ng/ml). This indicated that exogenously added Tat protein can activate the general transcriptional machinery in K562 cells, which suggested that Tat may act by directly increasing CBP function in conjunction with the effect of Tat in increasing CREB activation post-translationally. Thus, Tat may dictate a lineage commitment in K562 cells via increasing both CREB phosphorylation and chromosomal sequestration of the CREB-CBP complexes, which increases HAT activity.

Tat Treatment Increases CBP Coactivation Function via DNA Binding of Transcription Factors. In order to determine whether Tat mediates an increase in CBP function, we utilized a two-hybrid transfection strategy by using an expression vector coding for a chimeric protein

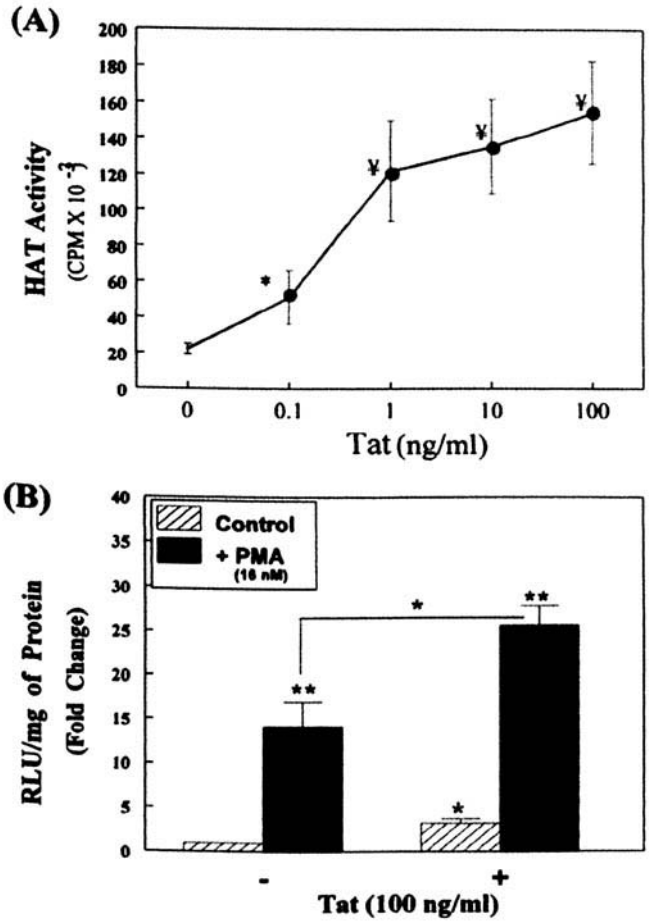

Figure 4. Effect of Tat on CBP coactivation-function. In (A), nuclear extracts were obtained from cells treated for 2 hrs with increasing concentrations of Tat (0.1–100 ng/ml), and HAT activity was monitored using ^{3}H-acetyl coenzyme-A and immobilized histone H-4 peptides. Studies were performed in four independent experiments ($n = 4$) and average fold changes in HAT activity (cpm $\times 10^{-3}$) are shown in the line graph in (A). Error bars represent ± SEM of values and significant changes are represented as $^*P < 0.05$, and $¥P < 0.01$. In (B), a two-hybrid transfection assay was employed to monitor CBP coactivation of GAL4-binding domain. Cells were cotransfected with pCBP-GAL4 and pGal4-Luc. Cells were treated with Tat (100 ng/ml) alone or in combination with PMA (16 n*M*) for 24 hrs and luciferase activity was monitored. Bar graphs in (B) represent the average relative light unit (RLU) normalized to the protein content in cell extracts (RLU/mg of protein). Data obtained from three independent experiments ($n = 3$) performed in quadruplicates are shown with the ± SEM of values. Significant changes from unstimulated controls are represented as *P* values ($^*P < 0.05$ and $^{**}P < 0.01$).

containing the CBP transactivation domain and a GAL-4 DNA-binding domain (pCBP-GAL4). To monitor CBP-directed gene expression we used a luciferase reporter vector under the transcriptional control of a GAL4 responsive element (pGAL4-Luc). Using this cotransfection strategy we monitored CBP-mediated gene expression in the presence or absence of Tat (100 ng/ml), or PMA (16 n*M*), or both (Fig. 4B). Transcriptional induction by CBP follows binding of the fusion protein to the GAL4 response element and activation can be measured by luciferase activity or relative light units (RLUs). The data have been normalized to the protein content in each sample (RLU/mg protein) and are shown as fold changes in luciferase activity. Exogenously added Tat protein (100 ng/ml) increased CBP-mediated transcriptional activity at least by 2-fold (hatched bars) over the untreated control cells. PMA stimulation alone at 16 n*M* showed a significant increase by approximately 10-fold, which was further increased to approximately 25-fold following cotreatment with both PMA and Tat (solid bars). Thus, the two-hybrid assay system showed that Tat treatment itself can increase the coactivation function of CBP, and Tat cotreatment can potentiate the effect of PMA. This increase in coactivator function may be partially involved in the increased HAT activity observed in Tat-stimulated cells. Tat seems to affect CREB activation by increasing CBP sequestration to the DNA binding domains. However, the limited amounts of CBP found in cells may not be enough to cause changes in lineage-specific gene expression following Tat treatment alone.

Tat Treatment Acutely Increases CBP Expression in K562 Cells. Because the inductive effect of Tat on CBP-associated HAT activity (Fig. 4A) and CBP-mediated gene expression (Fig. 4B) may be due to both transcriptional and post-translational increases in CBP levels, we monitored the effect of Tat on CBP expression in K562 cells. CBP message level was monitored by semiquantitative RT-PCR (Fig. 5A), and CBP protein level was assessed by Western immunodetection (Fig. 5B). RT-PCR analysis revealed that Tat (100 ng/ml) exposure can cause a rapid and transient increase in CBP mRNA levels, which was evident by a 3- to 4-fold increase within 3 hrs following Tat stimulation. The Tat-mediated increase in CBP mRNA levels peaked around 6 hrs after treatment, when a 6- to 8.5-fold enhancement over the basal level (0 time point) was observed. However, this increase in CBP gene expression was transient and returned to basal levels within 24 hrs. Western immunodetection of CBP protein levels similarly showed an increase of approximately 5- to 7-fold following 12 hrs of stimulation with 100 ng/ml of Tat (Fig. 5B). PMA (16 n*M*) stimulation of K562 cells did not, interestingly, show a detectable increase in CBP protein levels, and costimulation with Tat and PMA in fact resulted in lower amounts of CBP proteins compared with cells stimulated with Tat alone. These data revealed another potential mechanism by which exogenously added Tat protein may dictate lineage-specific signaling events and facilitate megakaryocytic differentiation in K562 cells.

Discussion

The present studies contribute to the growing body of evidence demonstrating the effects of exogenously added Tat protein in regulating gene expression in different cell types (22, 33–40). The paracrine actions of Tat have implicated a crucial role for this viral protein in precipitating HIV-1–induced hematologic dysfunctions (12, 15, 16, 19–22, 24). We had previously shown that Tat can suppress

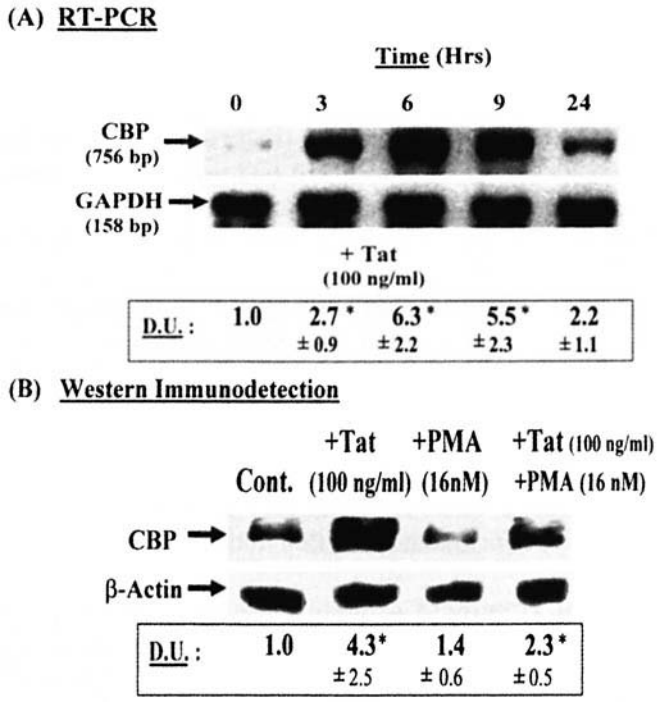

Figure 5. Temporal effects of Tat on CBP gene expression and protein levels. In (A), the effect of Tat on CBP message levels was monitored by RT-PCR. Total RNA was extracted from cells exposed to 100 ng/ml Tat protein for different time intervals (0, 3, 6, 9, and 24 hrs) and both CBP and GAPDH mRNA levels were determined using a semiquantitative RT-PCR assay. Fold changes in CBP product intensities are shown in empirical densitometric units (D.U.) following normalization to values obtained with the respective GAPDH-specific products in each sample. The D.U. values ($n = 3$) along with ± SEM are shown below the gel picture. In (B), Western immunodetection of CBP was carried out using extracts from cells 12 hrs after stimulation with either Tat (100 ng/ml), or PMA (16 n*M*), or both. The Western immunoblots were incubated with either CBP- or β-actin-specific antibodies. Band intensities were monitored using a densitometer and were normalized to β-actin in respective samples. Fold changes in CBP protein levels are represented in empirical D.U. ($n = 3$). Significant changes from unstimulated controls are represented as *P* values ($^*P < 0.05$).

butyric acid–induced erythroid differentiation of the K562 cell (38) and our recent studies depict that Tat may enhance the parallel pathway of megakaryocytic differentiation of these cells. During thrombopoietin-stimulated megakaryocytic differentiation of HPCs, increased cell surface expression of CD61 suggest that CREB is an early determinant of this pathway (49). We observed that Tat treatment increased CD61 expression in PMA-stimulated K562 cells (Fig. 1) and Tat treatment alone can increase CREB phosphorylation and DNA binding (Fig. 2). Flow cytometric analysis of cell surface CD61 expression showed a 3- to 4-fold higher level of CD61 expression in cells stimulated with Tat (100 ng/ml) and PMA (16 n*M*) compared with those stimulated with PMA alone. The role of Tat in suppressing megakaryocyte-specific colony formation (CFU-Meg) had been implicated in previous studies (20); this suppressive effect was shown to occur indirectly via TGF-β1 expression by cocultured macrophages. Our data with CREB transcription factor activation by Tat alone clearly suggest that Tat cotreatment increases the early megakaryocytic commitment steps in K562 cells. Although we have not shown a direct correlation between the Tat-mediated activation of CREB and CBP interactions in Tat-induced megakaryocytic differentiation, our studies indicate that Tat regulates the function of lineage-specific transcription factors, which may play a direct role in the differentiation commitment of K562 cells during early stages following stimulation.

Signaling pathways involved in either the erythroid or megakaryocytic differentiation of HPCs have been documented (64–66). In Jurkat cells and in PBMCs, Tat treatment can enhance ^{133}Ser-CREB phosphorylation and CRE-directed transcription (64). Our experiments in K562 cells similarly showed that Tat treatment alone can increase ^{133}Ser-Phospho-CREB levels and increased transcription factor binding to the CRE oligonucleotides (Fig. 2). A direct and rapid increase in CREB activation in K562 cells was observed within 60–90 mins following Tat treatment, suggesting that Tat may, at least in part, enhance the signaling required for the increase that led to commitment toward megakaryocytic differentiation. In our studies, although Tat treatment enhanced CREB phosphorylation and DNA binding (Fig. 2), transient transfection assays did not show a significant increase in CRE-directed transcription in the absence of PMA stimulation (Fig. 3A), which could be circumvented by overexpression of CBP in these cells (Fig. 3B) in which Tat alone was able to increase CRE-directed transcription. In addition, parallel signaling pathways may be necessary to form a productive transcription complex, chromatin binding, and increased HAT activity. Indeed, the cotransfection experiments in K562 cells overexpressing CBP (Fig. 3B) as well as our two-hybrid assays (Fig. 4) suggest the above possibilities. Tat protein increased nuclear HAT activity (Fig. 4A) and enhanced CBP-mediated transcriptional function (Fig. 4B). It is possible that Tat-induced CREB activation at the post-translational level may not be sufficient for megakaryocyte lineage-specific gene expression of CD61. The requirement of PMA stimulation and the facilitative effects of Tat on PMA-induced signaling suggest that the availability of transcriptional coactivators, as well as the concentrations of CREB transcription factors in close proximity with Tat and CBP, may also dictate the effects of Tat on lineage-specific gene expression and may directly contribute to the dysregulation of hematopoietic differentiation in HIV-1–infected individuals.

Early events dictating CREB function are regulated by post-transcriptional modifications of the cytoplasmic proteins. Phosphorylation of the serine residue at position 133 of CREB (^{133}Ser-P CREB) facilitates its interaction with the coactivator CBP (53), and subsequently, CREB binding to the cognate DNA binding site (61). Exogenously added Tat may not have a potent effect in enhancing CREB-directed transcription or in increasing megakaryocytic differentiation, possibly due to a limited amount of available CBP. In

K562 cells transfected with pCMV-CBP, a potent increase in CREB-directed gene expression was evident with 100 ng/ml of Tat alone and did not require PMA stimulation. This synergistic increase in CREB function may be an effect of Tat on both CREB activation at the post-translational level (e.g., phosphorylation) as well as an enhancement in DNA binding and facilitation of the transcriptional apparatus.

A possible explanation for the requirement of PMA to manifest the effects of Tat may be that CBP phosphorylation via protein kinase C (PKC) increases its coactivation function (52–55). As such, Tat may have to compete for a limited pool of phosphorylated CBP and thereby not allow CBP-mediated gene activation. Even though Tat can increase CBP function when the transactivation domain is fused to the GAL4 DNA binding domain (Fig. 4B), the availability of CBP may be a determining factor in the sequestration of the coactivator to the transcription complex. This would explain why PMA, a potent activator of PKC and consequently the MAP kinase pathway, which is inhibited by PD98059, may unmask Tat's inductive effects on CBP and its association with the activated/phosphorylated CREB transcription factor. Our studies with PD98059 showed a direct role for the MAP kinase pathway in CREB phosphorylation by Tat. It remains to be seen whether Tat-induced HAT activity or functional enhancement of CBP (or both) may also be linked to this or a parallel PKC-stimulated signaling cascade.

The potent, inductive effect of Tat that we have observed on CBP gene expression (Fig. 5A) and protein levels (Fig. 5B) is a novel finding. Previous studies have, however, shown that Tat inhibits CBP activity and may alter the selectivity of coactivators (67). We believe that this inhibitory effect observed in earlier studies may be due to the use of higher concentrations of Tat, which may compete with histones for CBP binding to the chromosomal loci. Compared with our experiments, a 10- to 100-fold higher concentration of Tat protein was used in those studies. In addition, the 101–amino acid isoform of HIV Tat was utilized in the previous study, whereas our experiments utilized the 86–amino acid isoform of Tat. The authors had indeed indicated that the additional 15–amino acid sequence is necessary for Tat-induced suppression of CBP activity to occur. Previous studies have shown direct interactions of Tat with CBP, which is known to modulate CBP activity (51). A number of different stimuli can also enhance CBP activity and coactivator association with transcription factors. However, these events are attributed to post-transcriptional modifications of CBP such as phosphorylation and acetylation (54, 56). Post-translational modification of transcription factors has also been shown to enhance their association with CBP/p300, such as that observed with the hematopoietic transcription factor GATA-1 (50, 71). CBP can also regulate the activity of transcription factor complexes by acting as a "molecular scaffold" on which CBP binds to several different transcription factors at the same time, thereby bringing them in close proximity and facilitate their interactions with the chromatin (56, 58). PMA stimulation of cells did not, interestingly, increase either CBP gene expression (data not shown) or CBP protein levels (Fig. 5B), and costimulation with PMA decreased Tat-induced effects on CBP protein levels. Previous observations have indeed shown that PKC stimulation causes post-translational modifications in the coactivators CBP/p300 but does not effect its gene expression (68). However, the potent increase in HAT activity (Fig. 4A) observed even with the lower concentrations (<1 ng/ml) suggested a partial contribution of CBP in Tat-mediated chromatin disruption, and implicated the involvement of other coactivators as well. Distinct functional differences between the HAT activity associated with p300 and CBP has been documented during retinoic acid–induced differentiation of mouse F9 embryonal carcinoma cells (72). Cumulatively, our studies showed that Tat treatment of K562 cells increases CBP levels, as well as CBP function and CBP interaction with CREB, which in conjunction with the acute post-translational effects of Tat on CREB phosphorylation and DNA binding, may ultimately result in enhanced megakaryocytic lineage commitment.

Tat may also be able to dysregulate differentiation via other lineages (73). Recent studies have indeed demonstrated that the cAMP-dependent pathway can negatively regulate erythroid differentiation (74). Furthermore, megakaryocyte-specific expression of the ganglioside-GM3 synthase gene in PMA-stimulated K562 cells involves the activation of CREB (75). Although we have not shown a direct correlation between the Tat-mediated activation of CREB and CBP in increasing PMA-induced megakaryocytic differentiation, extrapolation of the above findings suggest that this molecular pathway may be involved in increasing the megakaryocytic commitment to Tat-exposed HPCs.

K562 cells have been used by a number of previous studies as a comparative model to evaluate erythroid and megakaryocyte differentiation-specific signaling in CD34+ HPCs (76, 77). The Ras/extracellular-regulated kinase (ERK)–dependent pathway has been implicated in both erythroid (76) and megakaryocytic differentiation of K562 cells (77). Similar signaling cascades (e.g., ERK and p38), have also been shown to be involved in thrombopoietin-induced megakaryocytic and erythropoietin-induced erythroid differentiation of primary HPCs (78). Furthermore, K562 cells were used because we had previously documented the negative effects of Tat on erythroid differentiation (38). In a recent study, we observed that at 24–72 hrs poststimulation, a more significant increase in PMA-induced megakaryocytic differentiation was evident from an increase in both lineage-specific function (e.g., serotonin uptake) and gene expression (e.g., CD61 and TGF-β1) in Tat-treated K562 cells (79). Hence the use of K562 to observe the actions of Tat on lineage-specific differentiation

may elucidate the molecular mechanisms involved in CD34+ HPCs.

Although the use of 86 amino acid–containing Tat protein in our experiments may dampen the *in vivo* relevance of our findings, this Tat variant generated due to a mutation in the laboratory contains both the basic domain essential for HIV-1 long terminal repeat transactivation and the "RGD" motif reported to have extracellular effects via integrin-mediated signaling, and has been used in a number of previous published studies to delineate the molecular mechanism of action of Tat protein. Hence, data obtained with Tat-86 may be similar to data observed with the full-length Tat-100, and our observations may facilitate the understanding of megakaryocytic dysfunctions in individuals with HIV-1 infection. We speculate that this biological function of Tat may help increase HIV-1 infectivity in progenitor cell reservoirs by generating more replication-competent cells. Indeed, HPCs committed toward megakaryocytic pathways are found to be infectable by both X4 and R5 strains of HIV-1 (27–32). The resultant dysregulation in hematopoiesis may also play a role in manifesting the anemia and thrombocytopenia frequently observed in patients with AIDS (16, 22, 38). Our investigations reveal a putative molecular mechanism linked to Tat-induced effects on megakaryocyte lineage commitment in K562 cells. Tat protein may dictate differentiation-specific signaling in HPCs via regulating both early and late events in megakaryocytic differentiation, via regulating coactivator availability, and via their association with lineage-specific transcription factors.

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