CD28-dependent HIV-1 Transcription Is Associated with Vav, Rac, and NF-κB Activation*

Activation of HIV-1-infected T cells through the T cell receptor and costimulatory molecule CD28 induces proviral transcription; however, the mechanism behind this enhanced virus expression is unknown. Jurkat T cells and primary CD4+ T cells expressing a CD8α/CD28 chimeric receptor containing a mutation at tyrosine 200 in the cytoplasmic tail were unable to fully induce HIV-1 proviral transcription in response to CD8α/28 receptor cross-linking in comparison to CD28 costimulation. The loss of transactivation seen with the mutant chimeric receptor correlated with a decrease in Vav tyrosine phosphorylation. CD28-dependent activation of HIV-1 transcription also required the GTPase activity of Rac1, which was not activated during costimulation with the mutated receptor. Furthermore, the mutated receptor was unable to induce NF-κB DNA binding or transactivation, as demonstrated by electromobility shift assays and HIV-1 long terminal repeat and NF-κB-dependent reporter constructs. These studies show that signaling events initiated by tyrosine 200 of CD28 are required for efficient expression of HIV-1 transcription in activated T cells.

Engagement of the T cell receptor (TcR) and costimulatory molecule CD28 results in T cell proliferation, differentiation, and induction of cytokine expression, including IL-2. Human immunodeficiency virus type 1 (HIV-1) also utilizes T cell signaling events to ensure efficient virus transcription and regulation. Productive infection of CD4+ T cells by HIV-1 and efficient HIV-1 proviral transcription requires cell activation through the TcR and costimulatory molecules including CD28 (1–5). However, signaling through CD28 has been shown to inhibit HIV-1 infection by down-regulating chemokine receptor expression (6). This and other data suggests that CD28 serves as both a positive and negative regulator of HIV-1 infection and replication (1, 4, 5, 7–10). CD28 signaling is mediated by four tyrosine residues in its cytoplasmic tail, which when phosphorylated, recruit and activate various kinases, phosphatases, and adapter molecules including LCK, ITK, GRID, GRB2, MKP6, and PI3K leading to changes in IL-2 production as well as HIV-1 transcription (11–16). Deletion of all four tyrosine residues in the CD28 cytoplasmic tail results in a loss of its ability to enhance IL-2 production as well as HIV-1 transcription, demonstrating the necessity of these residues for proper signaling (17, 18). Tyrosine residues 188, 191, and 200 have been shown to positively regulate IL-2 production, but their effects on HIV-1 have not yet been examined (17). Tyrosine 173 has been shown to be involved in T cell activation, but how it influences IL-2 expression is not clear (19–24). We have shown that Tyr173, which recruits PI3K to the CD28 signaling complex, negatively regulates HIV-1 transcription by a Tat-dependent mechanism (18).

Signaling events initiated by the TcR and CD28 lead to increases in intracellular calcium, changes in cytoskeletal organization, and triggering of several kinase cascades. Activation of these signaling pathways targets various transcription factors including NF-κB, NFAT, AP-1, Sp1, and Ets-1 (25–28). NF-κB, NFAT, and AP-1, which have binding sites within the HIV-1 long terminal repeat (LTR), are induced by CD28 costimulation (26, 28–31). However, it is unclear which signals emanating from CD28 are responsible for the induction of these transcription factors and the activation of HIV-1 transcription.

One potential candidate is the guanine nucleotide exchange factor (GEF) Vav which has been shown to be targeted by CD28 signaling in the absence of TcR signaling (11, 15, 32, 33). Vav has multiple binding domains including SH2 and SH3 domains allowing it to serve as an adapter molecule for T cell signaling. It has also been suggested that Vav is essential for properly integrating TcR and CD28 signals (34). Negative regulation of Vav occurs through its interaction with cbl-b, which, upon CD28 ligation, releases Vav and is targeted for autoubiquitination and degradation (35). Previous studies have shown that downstream of CD28, Vav, in cooperation with Rac1, activates NF-κB (33, 36, 37).

Using Jurkat T cell lines and primary CD4+ T lymphocytes expressing chimeric CD8α/28 receptors with mutations in critical tyrosines located in the cytoplasmic tail, we have shown that CD28 signaling is required for efficient HIV-1 transcription (18). In this study, we specifically examine the role of Tyr200 in regulating HIV-1 transcription. We show that Tyr200 is necessary for efficient HIV-1 transcription in Jurkat T cells and primary CD4+ T cells through initiation of signaling events that enhance Vav phosphorylation, Rac1 activation, and NF-κB binding activity.

Julie A. Cook‡§, Lee Albacker§, Avery August§§, and Andrew J. Henderson¶¶

From the ‡Graduate Program in Biochemistry, Microbiology, and Molecular Biology, the §Department of Biochemistry and Molecular Biology, and the ¶¶Department of Veterinary Sciences, Pennsylvania State University, University Park, Pennsylvania 16802

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.

Published, JBC Papers in Press, July 3, 2003, DOI 10.1074/jbc.M302878200
CD28 Activation of HIV-1 Transcription

35813

EXPERIMENTAL PROCEDURES

Cell Lines and Primary Cells—Jurkat T cell line clone E6–1 obtained from ATCC were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.2 mM l-glutamine, and 0.5% Fungizone. 293T human embryonic kidney cells and Chinese hamster ovary (CHO) cells expressing FcR-II receptors (CHO-Fc) (gift from Dr. T. Millman, Yale University) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.2 mM l-glutamine, and 0.5% Fungizone. 293T cell lines overexpressing CD8α28 chimeric receptors 8WT and F200 were described previously (17, 18, 38). Expression of receptors was confirmed by flow cytometry. Primary blood lymphocytes were isolated from whole blood using a Ficoll-Histopaque (Sigma) gradient. Macrophages were separated by adherence to plastic overnight and CD4+ T cells were then positively selected from the non-adherent population using a CD4+ isolation kit (Dynal, Oslo, Norway).

Generation of HIV-1 Infectious Titors and Infections—Vesicular stomatitis virus glycoprotein (VSV-G) pseudotyped HIV-1 was generated by transfecting 293T cells with 15 μg of cDNA for either pNL4–3-Luc(+), ENV(–)–Nef(–)–HIV-luc (39) or pHRXbPL(+)–NEf(–)–HIV-PLAP (40); obtained from NIH AIDS Research and Reference Reagent Program, NIH, 3 μg of RSV-Rev, and 3 μg LTR VSV-G (41) by CaPO4 transfection (42). 293T transfection efficiency was assessed by determining luciferase activity using a Promega luciferase kit (Madison, WI). Supernatants were collected and filtered through a 0.45-micron disc prior to infection. Typically, the multiplicity of infection of infectious supernatants was 0.1–0.5. Cells were infected by culturing in the presence of virus stock for 12–24 h before replacing with fresh media. Cells were cultured for an additional 24 h before measuring luciferase activity to assess virus transcription. In some experiments, cells were infected with HIV-PLAP and 72-h post-infection HIV-PLAP positive cells were selected using CELLlection Pan Mouse IgG kit (Dynal, Lake Success, NY) along with anti-human PLAP antibodies (Sigma). Supernatants were collected and filtered through a 0.45-micron disc prior to infection. Typically, the multiplicity of infection of infectious supernatants was 0.1–0.5. Cells were infected by culturing in the presence of virus stock and 10 ng/ml PMA and 2 μg/ml PHA (Sigma) for 12–24 h before replacing with fresh media. Cells were cultured an additional 3 days prior to sorting with CD8 positive isolation kit (Dynal, Oslo, Norway). Sorted cells were allowed to rest overnight prior to activation.

Retroviral Exchange Assays—Cells were stimulated as described above for immunoprecipitations. Lysates were made according to Rac1 Activation Assay Kit (Upstate Technologies, Lake Placid, NY). Briefly, cells were resuspended in lysis buffer (MLB) supplemented with 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 μM sodium vanadate, and 1 μM sodium fluoride. Cell lysates were preincubated for 10 min with GST beads. Lysates were then incubated with PAK-1 PBD-agarose for 1 h at 4 °C. Beads were washed three times in MLB and samples were prepared for electrophoresis by adding 1× SDS loading dye. Samples were boiled for 5 min and resolved by 12% SDS-PAGE. Proteins were transferred to nitrocellulose membrane and GTP-bound Rac1 was identified by anti-Rac1 antibody.

Electrophoretic Mobility Shift Assays—Nuclear extracts from Jurkat and primary cells were prepared as previously described (43). Electrophoretic mobility shift assays (EMSA)s were performed by incubating 4 μg of protein from nuclear extracts with 4 μg of poly dIdC (Amersham Biosciences), 0.25 mM HEPES (pH 7.5), 0.6 μM KCl, 9.0 mM glyceral, 10 mM EDTA, 7.5 mM dithiothreitol, 50 mM MgCl2. Reaction mixtures were preincubated with 50-fold excess specific or nonspecific competitors, or 0.5 μg of polyclonal antibodies against NF-κB subunits or C/EBP for 20 min at room temperature. Samples were loaded onto a 6% polyacrylamide gel and electrophoresed at 120 V in 0.5× Tris borate-EDTA. Probes for EMSA were generated by annealing oligonucleotides representing HIV-1 LTR—5‘AGCTCCTGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCCCAGCGGAAAGTCCC...
CD38 Activation of HIV-1 Transcription

levels of CD3 stimulation alone (Fig. 1). Engagement of CD3, B did not result in induction of proviral transcription over the However, stimulating cells through CD3 and the F200 receptor

termic receptor plus CD3 showed induction of HIV-1 transcrip-
duress. Luciferase activity was assessed as a measure of
linked with CHO-Fc as described under “Experimental Proce-

Cells were then stimulated on CHO-Fc cells with 0.1 μg/ml anti-CD3 and 1.0 μg/ml anti-CD8α or anti-CD28 for 12–18 h and assayed for luciferase activity. Data are from a single experiment performed in triplicate, which is representative of a total of three experiments. C, expression of CD8α/28 chimERIC receptors in primary CD4+ T cells. Primary CD4+ T cells were isolated from whole blood and transiently transfected with 10 μg of HIV-luc and 20 μg of either pMHneo 8WT and pMHneo F200 and stained with FITC-conjugated isotype control (filled black line), or anti-CD8α F200 (broken black line) and analyzed by flow cytometry. Percentage of positive cells is noted in the legend. D, 24-h post-transfection, primary CD4+ T cells were stimulated on CHO-Fc cells with 0.1 μg/ml anti-CD3 and 1.0 μg/ml anti-CD8α or anti-CD28 for 12–18 h and assayed for luciferase activity. Data are the averages for three independent experiments performed in triplicate. Results were normal to the anti-CD3 plus anti-CD28 endogenous response, which was considered to be 100%.

and F200 cell lines were infected with an HIV-1 clone that included a luciferase reporter (HIV-luc) in place of the viral Nef gene (39). These cells were stimulated with different combinations of antibodies against CD3, CD8α, and CD28 and cross-linked with CHO-Fc as described under “Experimental Procedures.” Luciferase activity was assessed as a measure of proviral transcription. Cells stimulated through the 8WT chimeric receptor plus CD3 showed induction of HIV-1 transcription comparable to that observed when they were stimulated through the endogenous CD28 plus CD3 (Ref. 18, Fig. 1B). However, stimulating cells through CD3 and the F200 receptor did not result in induction of proviral transcription over the levels of CD3 stimulation alone (Fig. 1B). Engagement of CD3, CD8α, or CD28 alone did not efficiently activate viral transcription. These results suggest that tyrosine residue 200 in CD28 is necessary for efficient induction of HIV-1 transcription following CD3 and CD28 activation.

To determine whether these chimeric receptors were functional in primary T cells and mimic normal signals, 8WT and F200 receptors were transiently transfected with the HIV-luc cDNA into CD4+ T cells. Expression of the chimeric receptors was confirmed by flow cytometry with antibodies directed against CD8α (Fig. 1C). Receptors were stimulated with antibodies against CD3 plus CD28 or CD8α and cocultured with CHO-Fc for cross-linking. Activation through the wild type chimeric receptor resulted in induction of transcription that was comparable to that observed when endogenous CD28 was used as the costimulatory signal (Fig. 1D). However, cells transfected with the F200 mutant chimeric receptor and stimulated with anti-CD3 plus anti-CD8α were unable to induce HIV-1 transcription over background levels (Fig. 1D). These data show that the CD8α/28 chimeric receptors are functional in primary cells, as well as Jurkat cells, and suggest that Jurkat cells are a suitable model system for investigating the mechanisms of CD28 signaling. More importantly, results from both the primary CD4+ T cells and the Jurkat T cell lines demonstrate that Tyr200 is necessary for CD28-dependent HIV-1 activation.

Fig. 1. Tyr200 is required for CD8α-dependent HIV-1 transcription. A, expression of CD8α/28 chimeric receptors in Jurkat cells. Cells were stained with FITC-conjugated isotype control (filled line), anti-CD8α (solid black line), or anti-CD28 (broken black line) and analyzed by flow cytometry. B, Jurkat cells stably transfected with either 8WT or F200 CD8α/28 chimeric receptors were infected with HIV-luc for 18 h. Cells were then stimulated on CHO-Fc cells with 0.1 μg/ml anti-CD3 and 1.0 μg/ml anti-CD8α or anti-CD28 for 12–18 h and assayed for luciferase activity. Data are from a single experiment performed in triplicate, which is representative of a total of three experiments. C, expression of CD8α/28 chimeric receptors in primary CD4+ T cells. Primary CD4+ T cells were isolated from whole blood and transiently transfected with 10 μg of HIV-luc and 20 μg of either pMHneo 8WT and pMHneo F200 and stained with FITC-conjugated isotype control (filled line), anti-CD8α 8WT (solid black line), or anti-CD8α F200 (broken black line) and analyzed by flow cytometry. Percentage of positive cells is noted in the legend. D, 24-h post-transfection, primary CD4+ T cells were stimulated on CHO-Fc cells with 0.1 μg/ml anti-CD3 and 1.0 μg/ml anti-CD8α or anti-CD28 for 12–18 h and assayed for luciferase activity. Data are the averages for three independent experiments performed in triplicate. Results were normalized to the anti-CD3 plus anti-CD28 endogenous response, which was considered to be 100%.

Fig. 2. Tyr200 mediates Vav phosphorylation. 8WT or F200 cells were left untreated or stimulated with 0.1 μg/ml anti-CD3 and either 1.0 μg/ml anti-CD28 or 1.0 μg/ml anti-CD8α then cross-linked with 5 μg/ml goat anti-mouse (GAM) for 5 min. Protein A+G-agarose beads were used to pull-down Vav from whole cell lysates then probed for antiphosphotyrosine. Blots were reprobed with Vav specific antibodies. The blots shown are from two separate experiments, and the band intensities do not reflect differences in Vav levels in the individual cell lines.

Vav mediates guanine nucleotide exchange factor (GEF) with a molecular weight of ~95 kDa that has been shown to be phosphorylated post-CD28 ligation (15, 36, 45). To determine if phosphorylation of Vav was altered in F200 cells, immunoprecipitations were performed using 8WT and F200 cells either untreated or receiving costimulation through the endogenous CD28 or chimeric receptor. Cells activated with anti-CD3 plus anti-CD28 resulted in an increase in Vav phosphorylation over unstimulated cells. Costimulation through the SWT chimeric receptor resulted in an equivalent induction of Vav phosphorylation; whereas, cells receiving costimulation through the F200 chimeric receptor had significantly less phosphorylated Vav (Fig. 2). These data suggest that Vav is a critical downstream target of CD28 Ty200 and plays a role in the activation of HIV-1 transcription.

To confirm that Rac1, a downstream target of Vav, is a target of signaling through Ty200 we directly measured levels of activated GTP-bound Rac1. Cell lysates were prepared from 8WT and F200 cells following costimulation through CD28 or CD8α and a PAK PBD GST pull-down was performed to detect GTP-bound Rac1. Activation of the 8WT cells through CD3 and either CD28 or CD8α resulted in equivalent induction of GTP
CD28 Activation of HIV-1 Transcription

To induce HIV-1 transcription suggesting that NF-
H11002 dependent reporters. These deletion constructs still contained the
induction of the mutant NF-
H9260 B receptors following CD28 costimulation. Consistent with

NF-
H9260 B activation was assessed

NF-
H9260 B sites in the

NF-
H9260 B and SP1 binding sites. Mutation of the NF-
H9260 B sites in the

NF-
H9260 B-dependent reporters with both CD8α and CD28 stimulation but was not able
to induce the mutant NF-
H9260 B reporter (data not shown).

To confirm that NF-
H9260 B transcriptional activity was deficient in the F200 cells, NF-
H9260 B, and AP-1 reporters linked to luciferase were transiently transfected into the parental, SWT, and F200 Jurkat cell lines prior to T cell activation. As expected AP-1 and NF-
H9260 B-dependent reporters were induced by CD3 plus CD28 stimulation in the parental Jurkat cell line (Fig.

5A). The SWT cells were able to activate transcription of both NF-
H9260 B- and AP-1-dependent promoters when stimulated through CD3 plus CD28 or CD8α. The F200 cell line was unable to efficiently activate NF-
H9260 B- and AP-1-dependent reporters following CD3 plus CD8α stimulation, consistent with

NF-
H9260 B being upstream of AP-1 and NF-
H9260 B (Fig. 5, B and C). The deletion of AP-1 sites did not decrease the ability of the

−158 LTR reporter to respond to CD28 costimulation suggesting a minimal role for this factor in HIV-1 transcription. Therefore, we focused on the requirement of NF-
H9260 B in response to CD28 signaling.

Further characterization of NF-
H9260 B activation was assessed by electrophoretic mobility shift assays (EMSA) performed with nuclear extracts from SWT or F200 cells stimulated through CD3 and either endogenous CD28 or the chimeric CD8α receptor. In some experiments, cells were infected with HIV-PLAP, a recombinant HIV-1 clone that includes a placental alkaline phosphatase marker to positively select infected cells. This virus also encodes the viral protein Nef, which was replaced by the luciferase gene in the previous HIV-luc experiments. Nuclear extracts from HIV-1-infected SWT and F200 cells stimulated with antibodies against CD3 plus CD28 resulted in an increase in complex formation compared with unstimulated cells, which showed little to no binding activity.
8WT cells stimulated through CD3 and CD8α resulted in similar levels of NF-κB binding when compared with endogenous CD28 costimulation (Fig. 6A). In F200 cells stimulated with CD3 plus CD8α, no significant increase in complex formation was observed (Fig. 6A). Complexes formed in response to CD3 plus CD28 stimulation were competed away upon the addition of excess unlabeled NF-κB probe, but not with an irrelevant unlabeled competitor. Furthermore, these complexes were supershifted with antibodies specific to NF-κB (data not shown). Identical EMSA patterns were observed with extracts from F200 cells that were not infected, indicating that viral proteins are not altering NF-κB binding activity post CD3 plus CD28 stimulation (data not shown).

We also determined whether Tyr200 was necessary for NF-κB activation in primary CD4+ T cells by EMSA. CD4+ T cells isolated from whole blood were transduced with retroviral vectors carrying the 8WT and F200 chimeric receptors. Cells were sorted for CD8α expression and confirmed to be positive for chimeric receptors by flow cytometry. Populations from the sorted cells were ~90% positive for CD8α expression (data not shown). Sorted cells were stimulated with antibodies against CD3 plus CD28 or CD8α in the presence of CHO-Fc cells. 8WT cells induced similar NF-κB binding complexes following activation with anti-CD3 plus anti-CD28 or anti-CD8α (Fig. 6B). The F200 cells were able to induce NF-κB binding in response to CD3 plus CD28 signaling but no significant change in NF-κB binding activity was observed when costimulation was provided by the F200 chimeric receptor. The NF-κB binding observed in these cells was specific since unlabeled NF-κB probe but not unlabeled nonspecific probe competed for binding (Fig. 6B). These data confirm that NF-κB is downstream of Tyr200 and further implicates this pathway in regulating CD28-dependent HIV-1 transcription.

**DISCUSSION**

In this report, we demonstrate that residue Tyr200 of the CD28 receptor is required for the activation of HIV-1 transcription in both Jurkat and primary CD4+ T cells. We also show that a dominant negative Rac1 inhibits LTR-luc responses and that mutation of Tyr200 decreases Vav tyrosine phosphorylation and Rac1 activation. These two signaling molecules have been shown to regulate NF-κB activation, which is in agreement with a loss of NF-κB reporter and binding activity in the F200 cell line. The decrease in NF-κB activity severely impairs HIV-1 transcription, consistent with previous studies indicating that this is a critical transcription factor for HIV-1 LTR activity and virus replication (26, 31, 47).

The CD28 receptor is an important costimulatory molecule for T cell activation and has four tyrosine residues and a proline-rich domain, which are involved in the recruitment and activation of downstream effector molecules (16). Studies examining the induction of IL-2 and HIV-1 transcription have
shown that the activity and interplay of these tyrosine residues is complex and may act competitively or synergistically with one another (11, 17, 22, 33, 48). Tyr-173 has been identified as recruiting ITK, GRB-2, and PI3K to the receptor (11, 14, 15).

We have previously reported that mutating Tyr-173 or inhibiting PI3K activity increases HIV-1 transcription through a Tat-dependent mechanism (18). However, the contribution of Tyr-200 in activating HIV-1 transcription does not require viral-encoded proteins such as Tat or Nef, but mediates changes in cellular transcription factors (18). We have not yet determined the roles of Tyr-186 and Tyr-191 in HIV-1 transcription. Taken together our results demonstrate that the Tyr-173 and Tyr-200 residues recruit different signaling complexes to activate distinct pathways leading to either negative or positive signals to the HIV-1 LTR.

CD28 activation leads to an increase in Vav phosphorylation, which has been suggested to be mediated by tyrosine Tyr173 (52). In this report, Nef expressing viruses were used to demonstrate that this pathway is necessary for the CD28-dependent HIV-1 transcription. Therefore, T cell induction of HIV-1 transcription through Tyr-200 as suggested by the LTR-reporter constructs, which do not encode the viral proteins. These data taken together with other reports suggest that CD28 activation of Vav and Rac1 are critical steps in the activation of Tyr-200 (36, 37). However, additional experiments are required to establish a direct role for MEKK1 in events downstream of CD28 Tyr200. Experiments using chemical inhibitors against p38 and MEK1 have suggested that these MAPK pathways are not involved in CD28 Tyr200 mediated HIV-1 transcription (data not shown).

The viral protein Nef has been shown to influence T cell activation through interactions with several signaling intermediates downstream of the TcR and CD28. Vav is a potential target of Nef, although how this interaction with Vav influences HIV-1 transcription has not been investigated (54). Furthermore, Nef potentially alters NFAT transactivation but, its role in regulating NFAT expression has not been investigated (54). Furthermore, overexpression of Vav plus Rac1 or MEKK1 were able to circumvent CD3 plus CD28 signaling to induce NF-kB reporter activity (36, 45, 53). We confirmed that Rac1 is necessary for induction of the HIV-1 LTR and observe strong activation of the HIV-1 LTR when MEKK1 is constitutively active (data not shown). This would be consistent with reports that MEKK1 can activate NF-kB and may be involved downstream of Vav (36, 45, 53). However, additional experiments are required to establish a direct role for MEKK1 in events downstream of CD28 Tyr200.
48. Pages, F., Ragueneau, M., Klasen, S., Battifora, M., Couez, D., Sweet, R., Truneh, A., Ward, S. G., and Olive, D. (1996) *J. Biol. Chem.* **271**, 9403–9409
49. Klasen, S., Pages, F., Peyron, J. F., A. Cantrell, D., and Olive, D. (1998) *Int. Immunol.* **10**, 481–489
50. Raab, M., Da Silva, A., Findell, P. R., and Rudd, C. E. (1997) *Immunity* **6**, 155–164
51. Herndon, T. M., Shan, X. C., Tsokos, G. C., and Wange, R. L. (2001) *J. Immunol.* **166**, 5654–5664
52. Yoon, K., and Kim, S. (1999) *J. Gen. Virol.* **80**, 2951–2956
53. Tao, L., Wadsworth, S., Mercer, J., Mueller, C., Lynn, K., Siekierka, J., and August, A. (2002) *Biochem. J.* **363**, 175–182
54. Fackler, O. T., Luo, W., Geyer, M., Alberts, A. S., and Peterlin, B. M. (1999) *Mol. Cell* **3**, 729–739
CD28-dependent HIV-1 Transcription Is Associated with Vav, Rac, and NF-κB Activation
Julie A. Cook, Lee Albacker, Avery August and Andrew J. Henderson

J. Biol. Chem. 2003, 278:35812-35818.
doi: 10.1074/jbc.M302878200 originally published online July 3, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M302878200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 29 of which can be accessed free at http://www.jbc.org/content/278/37/35812.full.html#ref-list-1