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Human Immunodeficiency Virus Type 1 Nef Protein Inhibits NF-κB Induction in Human T Cells

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Human immunodeficiency virus type 1 (HIV-1) can establish a persistent and latent infection in CD4$^+$ T lymphocytes (W. C. Greene, N. Engl. J. Med. 324:308–317, 1991; S. M. Schnittman, M. C. Psallidopoulos, H. C. Lane, L. Thompson, M. Baseler, F. Massari, C. H. Fox, N. P. Salzman, and A. S. Fauci, Science 245:305–308, 1989). Production of HIV-1 from latently infected cells requires host cell activation by T-cell mitogens (T. Folks, D. M. Powell, M. M. Lightfoote, S. Benn, M. A. Martin, and A. S. Fauci, Science 231:600–602, 1986; D. Zagury, J. Bernard, R. Leonard, R. Cheynier, M. Feldman, P. S. Sarin, and R. C. Gallo, Science 231:850–853, 1986). This activation is mediated by the host transcription factor NF-κB [G. Nabel and D. Baltimore, Nature (London) 326:711–717, 1987]. We report here that the HIV-1-encoded Nef protein inhibits the induction of NF-κB DNA-binding activity by T-cell mitogens. However, Nef does not affect the DNA-binding activity of other transcription factors implicated in HIV-1 regulation, including SP-1, USF, URS, and NF-AT. Additionally, Nef inhibits the induction of HIV-1- and interleukin 2-directed gene expression, and the effect on HIV-1 transcription depends on an intact NF-κB-binding site. These results indicate that defective recruitment of NF-κB may underlie Nef's negative transcriptional effects on the HIV-1 and interleukin 2 promoters. Further evidence suggests that Nef inhibits NF-κB induction by interfering with a signal derived from the T-cell receptor complex.

Human immunodeficiency virus type 1 (HIV-1) can establish a latent infection in CD4$^+$ T cells (14, 29). Production of HIV-1 from latently infected cells requires host cell stimulation by T-cell mitogens (9, 34). Stimulation of T cells by T-cell-specific stimuli (e.g., antigen or antibody to CD2 or CD3) or nonspecific mitogens (e.g., phytohemagglutinin [PHA] and phorbol 12-myristate 13-acetate [PMA]) results in the induction of the DNA-binding activity of the host transcription factor NF-κB (14). The NF-κB family of proteins normally regulates the expression of genes involved in T-cell activation and proliferation, such as interleukin 2 (IL-2) and the alpha subunit of the IL-2 receptor (14). The HIV-1 promoter possesses two adjacent NF-κB-binding sites which allow the virus to subvert the normal activity of NF-κB to enhance its own replication (23).

Previous work suggests that the HIV-1-encoded Nef protein is a negative regulator of HIV-1 replication (1, 7, 20, 25, 31). Furthermore, we and others have found that Nef may suppress both HIV-1 and IL-2 transcription (1, 21, 25). To investigate whether Nef affects the DNA binding activity of NF-κB or other transcription factors implicated in HIV-1 regulation, we used human T-cell lines stably transfected with the *nef* gene. Jurkat (J25) human T-cell clone 133 constitutively expresses the NL43 *nef* gene. 22F6 cells represent another antibiotic-resistant clone of J25 cells; however, these cells do not contain *nef* sequences and do not express Nef (21). Additionally, we used oligoclonal Jurkat E6-1 and HPB-ALL cells expressing the SF2 *nef* gene either in the correct orientation (Jurkat/LnefSN and HPB-ALL/LnefSNS1 cells) or in the reverse orientation (Jurkat/LfenSN and HPB-ALL/LfenSN cells) with respect to the Moloney murine leukemia virus promoter (10). These cells represent a mixed population of cells expressing Nef to various degrees and were used to exclude the possibility that clonal selection accounts for Nef effects observed in the J25 clones.

To determine the impact of T-cell activation on the expression of Nef, the human T-cell lines were stimulated with PHA and PMA. Cells were maintained in logarithmic growth in RPMI 1640 medium supplemented with 10% fetal bovine serum and 2 mM glutamine. J25 and Jurkat E6-1 cells (5×10^6 each) and HPB-ALL cells (1.5×10^7) were either not stimulated or stimulated with 13 μg of PHA-P (Sigma) and 75 ng of PMA (Sigma) per ml for 4 h. The cells were lysed in RIPA buffer, and lysates were immunoprecipitated with rabbit anti-Nef polyclonal serum (6). The immunoprecipitates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% polyacrylamide), and the proteins were transferred to nitrocellulose for Western immunoblot analysis. The primary antibody was the rabbit anti-Nef serum, and the secondary antibody was alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin, specific for the heavy chain (Promega). The proteins were visualized by color development with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate toluidinium (Promega). Band intensity was determined by laser densitometry scanning of the Western blot and was in the linear range of analysis as established by a standard curve. Jurkat E6-1 cells were obtained from the AIDS Repository, American Type Culture Collection (Arthur Weiss) (32), and were stably transduced with the SF2 *nef* gene as previously described (10).

Immunoblot analysis with anti-Nef antibodies showed that

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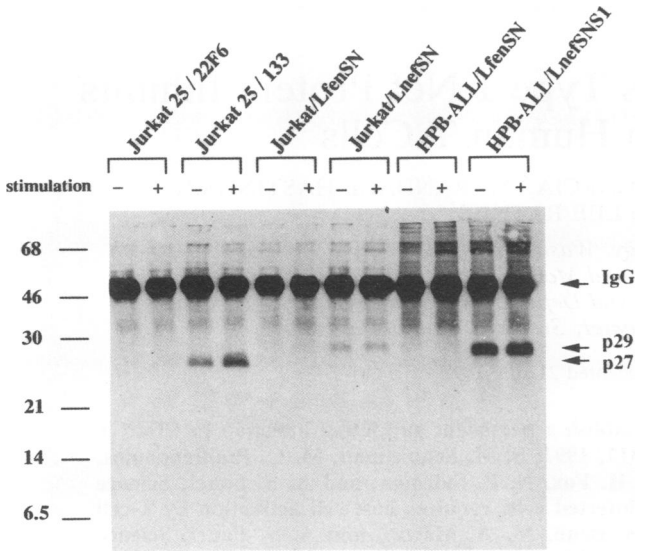

FIG. 1. Immunoblot analysis of the HIV-1 Nef protein in stably transfected and transduced human T-cell lines. Cell lysates were immunoprecipitated with rabbit anti-Nef polyclonal serum, electrophoresed, transferred to nitrocellulose, and immunoblotted with the same anti-Nef serum. The cells were either unstimulated (−) or stimulated (+) with PHA and PMA before cell harvesting. Prestained protein size markers are indicated on the left in kilodaltons. Nef protein in the 133 cells (21) was expressed from the *nef* gene of isolate pNL432 and had an apparent molecular mass of 27 kDa, whereas the Nef proteins expressed in the Jurkat E6-1 and HPB-ALL cells were encoded by the *nef* gene of isolate SF2 and demonstrated an apparent molecular mass of 29 kDa. Immunoglobulin G (IgG) heavy chain, which was present in the antiserum used for the immunoprecipitation step, is indicated at the right.

stimulation caused a two- to threefold increase in Nef expression in clone 133 cells (Fig. 1). This increase was probably due to the inducibility of the chimeric simian virus 40 (SV40)–human T-cell leukemia virus type I promoter used to direct Nef expression. However, Nef expression was not induced in the Jurkat E6-1 or HPB-ALL cells (Fig. 1). The level of Nef expressed in these cells is comparable to the amount of Nef generated by HIV-1 in productively infected CEM human T cells (data not shown). The difference in the apparent molecular weight of the Nef produced in clone 133 cells and those produced in the Jurkat/LnefSN and HPB-ALL/LnefSNS1 cells is due to the presence of an alanine at amino acid position 54 in the NL43 Nef gene compared with the presence of an aspartic acid at that position in the SF2 Nef gene (26). The amount of lysate equivalents loaded in the HPB-ALL/LnefSNS1 lanes was threefold larger than that in the Jurkat/LnefSN lanes. Nevertheless, the amount of Nef expressed in the HPB-ALL/LnefSNS1 cells was approximately fourfold larger than the amount produced in the Jurkat/LnefSN cells (Fig. 1). Nef did not appear to be toxic, in that the Nef-producing cells exhibited the same doubling time and morphology as the control cells.

Gel shift assays were performed with nuclear extracts prepared from stimulated and unstimulated cells. Nuclear extracts were prepared from 5×10^7 cells with a modified version of the method of Dignam et al. (8) as adapted by Montminy and Bilezikjian (22). Following ammonium sulfate precipitation, nuclear proteins were resuspended in 100 μl of a solution containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.9), 20 mM KCl, 1 mM $MgCl_2$, 2 mM dithiothreitol, and 17% glycerol (33) with the addition of 10 mM NaF, 0.1 mM sodium vanadate, and 50 mM beta-glycerol-phosphate. Cytoplasmic extracts consisted of the supernatant resulting from the lysis of cells in hypotonic lysis solution, Dounce homogenization, and low-speed centrifugation to pellet nuclei. Binding reaction mixtures contained 2 μl (2 μg) of nuclear extract (Fig. 2a through d) or 6 μl (7 μg) of cytoplasmic extract (Fig. 2e), 2 μg of poly(dI-dC) (Pharmacia), 100-fold molar excess of unlabeled NF-κB mutant oligonucleotide (ACAACTCACTTTCCGCT GCTCACTTTCCAGGGA), and 20,000 cpm of end-labeled oligonucleotide probe, in DNA binding buffer (27), in a final volume of 22 μl. Reactions were performed at 30°C for 25 min, immediately loaded on a 4.5% polyacrylamide gel with 0.5× Tris-borate-EDTA, and run at 200 V. Oligonucleotides used were as follows: NF-κB, ACAAGGGACTTTCCGC TGGGACTTTCCAGGGA; SP-1, CAGGGAGGCGTGGCC TGGGCGGGACTGGGGAGTGGCGTCC. All DNA probes were gel purified and end labeled with [γ-^{32}P]ATP. The intensity of the indicated bands was determined by laser densitometry and by measuring the radioactivity of excised bands in a liquid scintillation counter. There was a linear relationship between the amount of extract used and DNA-binding activity (data not shown). There was no NF-κB DNA-binding activity with the cytoplasmic extracts in the absence of deoxycholic acid (data not shown). Protein concentration was determined with the Bradford reagent (Bio-Rad) with bovine serum albumin as a standard. Nuclear extract preparations and binding reactions were repeated on three separate occasions with similar results.

The induction of NF-κB activity in stimulated 133 cells was suppressed five- to sevenfold compared with that in the 22F6 cells. This inhibition was evident 40 min poststimulation and was sustained throughout the 4-h stimulation period (Fig. 2a). J25 clone 22D8 cells represent a distinct clonal cell line which, like the 133 cells, also stably express Nef (21). NF-κB induction was suppressed four- to fivefold in the 22D8 cells compared with that in the 22F6 cells (Fig. 2b). NF-κB suppression was more profound in the 133 cells than in the 22D8 cells, which correlates with the observation that Nef expression was higher in the 133 cells (21). Similarly to the Nef-expressing J25 clones, Nef inhibited NF-κB induction three- to fourfold in the Jurkat/LnefSN and HPB-ALL/LnefSNS1 cells compared with their non-Nef-expressing counterparts (Fig. 2c and d). Nef-mediated NF-κB suppression was more profound in the Jurkat/LnefSN cells than in the HPB-ALL/LnefSNS1 cells, even though the HPB-ALL/LnefSNS1 cells expressed severalfold higher levels of Nef. This result is likely due to the biological differences that exist between the two cell lines. That is, Jurkat cells may be more sensitive to the effects of Nef than HPB-ALL cells because of differential expression of proteins involved in signal transduction. That Nef-mediated NF-κB suppression in the 133 and 22D8 cells was greater than in the Jurkat/LnefSN and HPB-ALL/LnefSNS1 cells may be due to the expression of a different *nef* allele in the 133 and 22D8 cells. Alternatively, this result could be due to the fact that every cell in the culture of 133 and 22D8 cells produced a relatively high level of Nef, whereas the Jurkat/LnefSN and HPB-ALL/LnefSNS1 cells represent a mixed population of cells expressing low and high levels of Nef or no Nef at all.

NF-κB activity in nuclei from unstimulated cells was extremely low but detectable, and no differences between the Nef-expressing and control cells were observed (data not shown). Additionally, when cytoplasmic extracts from un-

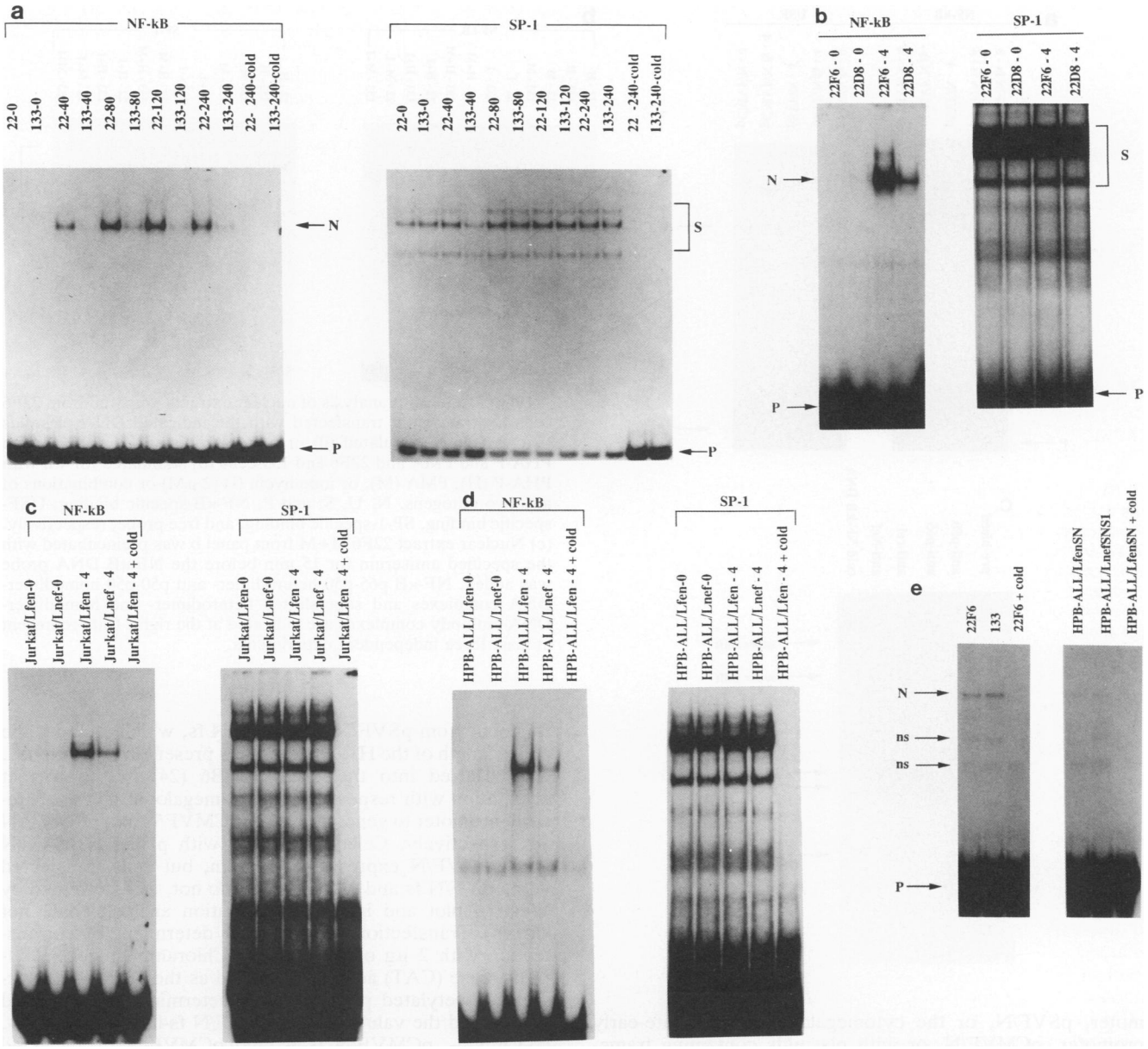

FIG. 2. Gel shift analysis of NF-κB activity in nuclear extracts prepared from J25 (a and b), Jurkat E6-1 (c), or HPB-ALL (d) cells. (a) 22F6 and 133 cells were stimulated with PHA (13 μg/ml) and PMA (75 ng/ml) for 0, 40, 80, 120, or 240 min; 22F6 and 22D8 (b), Jurkat E6-1 (c), or HPB-ALL (d) cells were not stimulated (0) or were stimulated with PHA and PMA as described above for 4 h (4). DNA probes used for binding are specified on the top of each panel. (e) Cytoplasmic protein extracts (7 μg each) from the indicated cells were incubated with the NF-κB DNA probe as described in the text, in the presence of 0.6% deoxycholic acid (Sigma). N, S, and P, NF-κB-specific binding, SP-1-specific binding, and free probe, respectively. SP-1 binding served as a control for extract quality and specificity of Nef effects. Cold indicates that 100-fold molar excess of unlabeled DNA was added for competition. ns, nonspecific binding. Data represent at least three independent experiments.

stimulated cells were treated with deoxycholic acid (which releases NF-κB from its cytoplasmic inhibitor, IκB [3]), they exhibited NF-κB activity independent of Nef expression (Fig. 2e). Finally, that Nef suppressed the level of NF-κB induction after only 40 min of stimulation suggests that Nef does not suppress p110 or p65 NF-κB mRNA expression. These observations indicate that Nef affects the recruitment and not the cytoplasmic concentration of NF-κB. The binding of SP-1 was independent of Nef expression and stimulation, and the amount of SP-1 probe used in these gel shift assays was not limiting (Fig. 2a through d). In addition, no differences in binding to NF-AT-, USF-, and URS-specific probes between the 22F6 and 133 cells were observed (data not shown). These data suggest that Nef specifically inhibited the induction of NF-κB activity.

To further demonstrate Nef's suppressive effect on NF-κB recruitment, 22F6 cells were transiently transfected with DNA plasmids expressing Nef from the SV40 early pro-

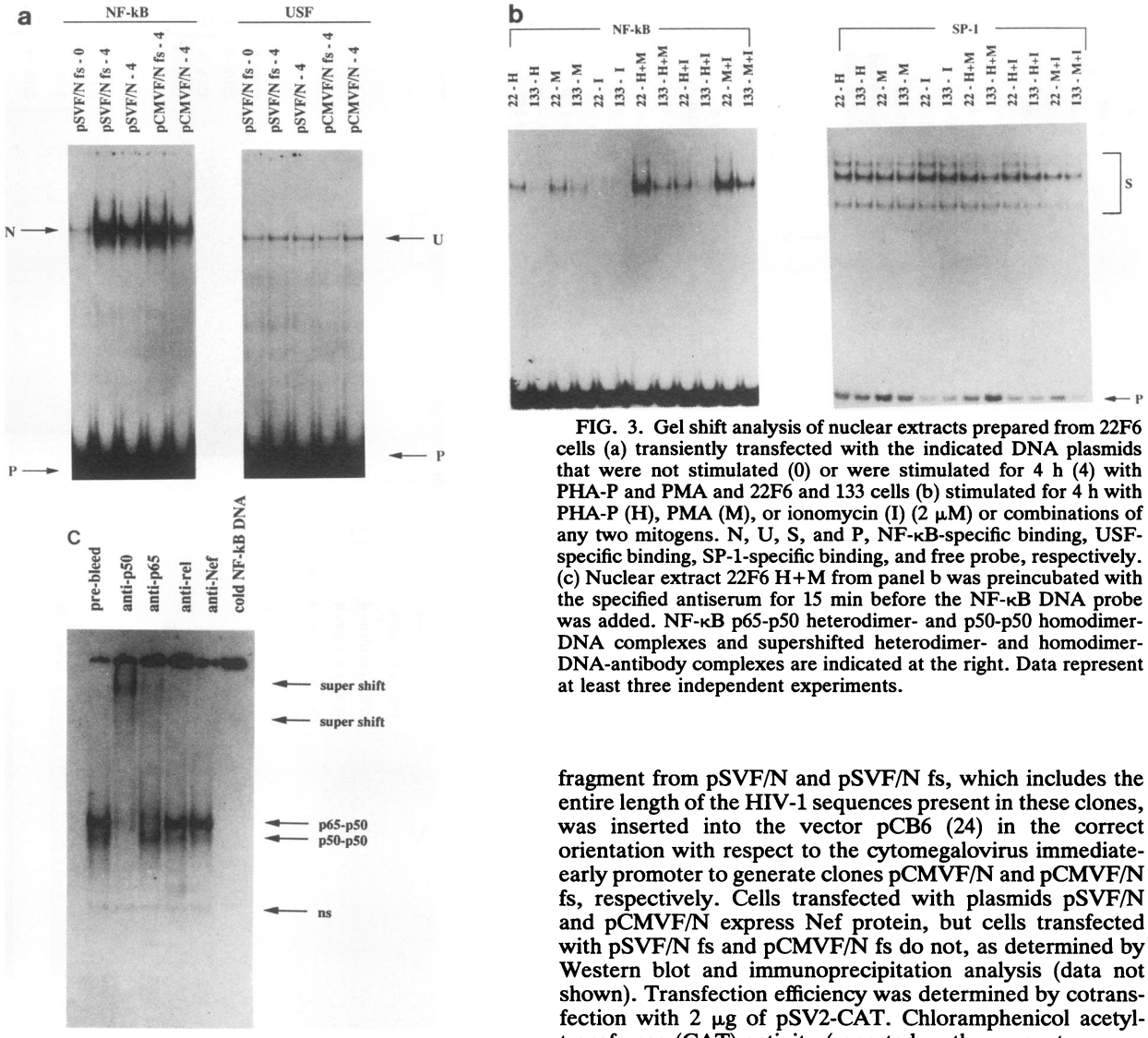

FIG. 3. Gel shift analysis of nuclear extracts prepared from 22F6 cells (a) transiently transfected with the indicated DNA plasmids that were not stimulated (0) or were stimulated for 4 h (4) with PHA-P and PMA and 22F6 and 133 cells (b) stimulated for 4 h with PHA-P (H), PMA (M), or ionomycin (I) (2 μM) or combinations of any two mitogens. N, U, S, and P, NF-κB-specific binding, USF-specific binding, SP-1-specific binding, and free probe, respectively. (c) Nuclear extract 22F6 H+M from panel b was preincubated with the specified antiserum for 15 min before the NF-κB DNA probe was added. NF-κB p65-p50 heterodimer- and p50-p50 homodimer-DNA complexes and supershifted heterodimer- and homodimer-DNA-antibody complexes are indicated at the right. Data represent at least three independent experiments.

moter, pSVF/N, or the cytomegalovirus immediate-early promoter, pCMVF/N, or with plasmids containing frameshift mutations in the *nef* gene (pSVF/N fs and pCMVF/N fs, respectively). Nuclear extract preparation and DNA-binding reactions were as described above. 22F6 cells (2×10^7) (Fig. 3a) were transfected with 30 μg of the indicated plasmid DNA by using DEAE-dextran. Briefly, cells (10^7) were incubated with plasmid DNA suspended in a solution containing 10 ml of serum-free RPMI 1640, 0.25 M Tris (pH 7.3), and 125 μg of DEAE-dextran (Sigma) per ml at 37°C for 40 min. Following centrifugation at 2,000 × *g* for 7 min, the cells were maintained in growth medium for 60 h prior to stimulation and cell harvesting. Plasmid pSVF/N is similar to plasmid pSVF (25), except that HIV-1 nucleotides 8994 to 9213 (including the NF-κB recognition sites) and 3′ flanking cellular sequences were deleted. Plasmid pSVF/N was digested at the unique *Bgl*II site at codon 88 of the *nef* gene, the sticky ends were filled in with the Klenow fragment of DNA polymerase I, and the plasmid was religated with T4 DNA ligase. This plasmid was called pSVF/N fs to indicate the introduction of a frameshift in the *nef* gene. The *Bam*HI fragment from pSVF/N and pSVF/N fs, which includes the entire length of the HIV-1 sequences present in these clones, was inserted into the vector pCB6 (24) in the correct orientation with respect to the cytomegalovirus immediate-early promoter to generate clones pCMVF/N and pCMVF/N fs, respectively. Cells transfected with plasmids pSVF/N and pCMVF/N express Nef protein, but cells transfected with pSVF/N fs and pCMVF/N fs do not, as determined by Western blot and immunoprecipitation analysis (data not shown). Transfection efficiency was determined by cotransfection with 2 μg of pSV2-CAT. Chloramphenicol acetyltransferase (CAT) activity (reported as the percent conversion to acetylated products) was determined as described below, and the values for the pSVF/N fs-0-, pSVF/N fs-4-, pSVF/N-4-, pCMVF/N fs-4-, and pCMVF/N-4-transfected cells were 51, 60, 61, 58, and 61%, respectively. A USF-specific DNA probe (corresponding to nucleotides −159 to −173 of the HIV-1 long terminal repeat, GCCGCTAG CATTTCATCACGTGGCCCGAGAGCTGC) was used as a control for the specificity of Nef effects and extract integrity.

NF-κB induction was consistently inhibited at least twofold in cells transfected with either pSVF/N or pCMVF/N compared with cells transfected with their *nef* mutant counterparts (Fig. 3a). Transfection efficiencies in these experiments were determined by cotransfecting cells with the pSV2-CAT plasmid and measuring CAT activity. No significant differences in transfection efficiency between the *nef*-expressing and the *nef* mutant plasmids were observed (Fig. 3a). The suppressive effect of Nef in these transiently transfected cells was not as dramatic as the effects observed in the stably transfected and transduced cells. The more subtle effect of Nef in this experiment may be due to the expression of a *nef* allele which was derived from an HIV-1 isolate distinct from either the NL-43 or the SF2 isolates

(25). In addition, cells which did not receive the *nef* expression plasmid during the transient-transfection process were not eliminated (by antibiotic selection) from the total cell population.

To explore the relative contributions of individual mitogens to the recruitment of Nef-inhibitable complexes, cells were stimulated with PHA, PMA, or ionomycin alone or in combination. The maximal induction of NF-κB activity occurred when PHA was combined with PMA (Fig. 3b). This result, coupled with the observation that PHA mimics the effects of the natural ligand for the T-cell receptor (TCR) complex (32), suggests that Nef may inhibit signal transduction emanating from the TCR complex. The addition of the Ca^{2+} ionophore, ionomycin, when coupled with PMA treatment, partially substituted for the absence of PHA with respect to NF-κB induction (Fig. 3b). However, ionomycin treatment did not significantly reduce Nef's inhibitory effects, suggesting that events other than Ca^{2+} mobilization may be disrupted by Nef.

Using antibodies against the p50 and p65 NF-κB subunits, we found that Nef-inhibitable complexes included both p50-p50 homodimers and p50-p65 heterodimers (Fig. 3c). Anti-p50, anti-p65, anti-v-*rel*, and prebleed sera (Fig. 3c) were kindly provided by Mark Hannink (University of Missouri, Columbia, Mo.). Because the gels in Fig. 3a and b and Fig. 2 were run for a shorter length of time, the two bands indicated in Fig. 3c appear as one band in Fig. 3a and b and Fig. 2.

To determine whether Nef-mediated inhibition of NF-κB-binding activity correlated with a decrease in transcriptional activity, cells were transfected with DNA plasmids which use the HIV-1 long terminal repeat to direct expression of a heterologous gene product, CAT. Jurkat cells were transfected, as described above, with 15 μg of the CAT constructs indicated in Fig. 4. Following transfection, the cells were maintained in growth medium for 24 h. Cells were or were not treated with PHA-P (13 μg/ml) and PMA (75 ng/ml) and incubated for an additional 18 h. Cell extracts were prepared, and CAT activity was assessed by standard methods (13). Extract equivalent to 3×10^6 cells was used for each 18-h reaction. CAT activity was in the linear range of analysis with respect to extract amount and incubation time (data not shown). CAT assays were normalized to a noninducible control plasmid, RSV-CAT (12) (2 μg), which was transfected in parallel with the HIV-1-CAT plasmids as described above. Assays were also normalized to protein concentrations as determined by Bradford reagent analysis (Bio-Rad). The amount of CAT activity was quantitated by excising the spots corresponding to the unacetylated and acetylated forms of [^{14}C]chloramphenicol and measuring radioactivity in a liquid scintillation counter. CAT activity is expressed as the percentage of radioactivity in the acetylated forms compared with the sum of that of the acetylated and unacetylated forms. The wild-type HIV-1-CAT (CD12-CAT was derived by a small deletion in the *nef* coding sequence upstream of the long terminal repeat start site of clone C15-CAT [2]), and mutant NF-κB HIV-1-CAT (23) and IL-2-CAT (30) plasmids were generously provided by Steven Josephs, Gary Nabel, and Gerald Crabtree, respectively. δNRE-HIV-1-CAT was generated by excising the *Ava*I-*Ava*I fragment from C15-CAT (2) and therefore lacks the negative regulatory element sequences present in C15-CAT.

CAT activity correlated well with DNA-binding activity in that 133 cells exhibited a capacity to induce CAT activity that was fivefold less than that of 22F6 cells (Fig. 4a). Similarly, CAT activity induction was suppressed twofold in

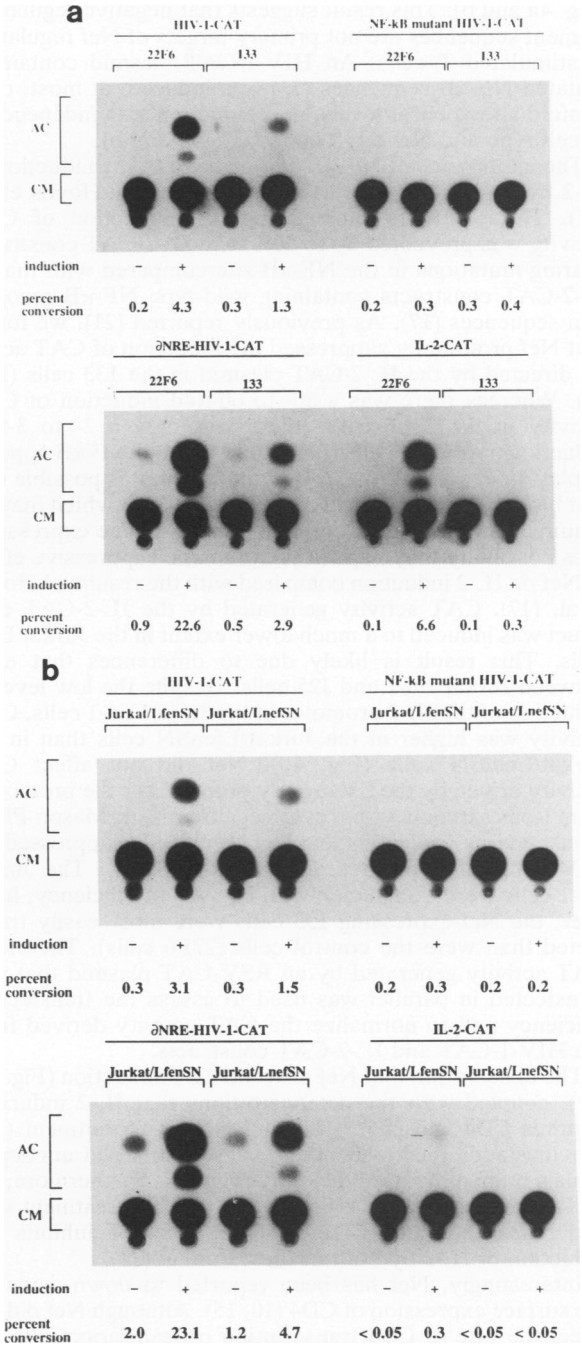

FIG. 4. CAT assays of extracts from cells transiently transfected with HIV-1-CAT and IL-2-CAT DNA plasmids. J25 (a) and Jurkat E6-1 (b) cells were transfected with the CAT constructs as indicated above each panel. Cells were not induced (−) or were induced (+) with PHA and PMA. CAT activity was determined by conversion of unacetylated [^{14}C]chloramphenicol (CM) to monoacetylated forms (AC). These data represent at least three independent experiments.

the Jurkat/LnefSN cells compared with that in the Jurkat/LfenSN cells (Fig. 4b). This inhibition was demonstrated with both wild-type HIV-1-CAT and the negative regulatory element deletion clone, δNRE-HIV-1-CAT, which lacks nucleotides −453 to −156 of the HIV-1 long terminal repeat

(Fig. 4a and b). This result suggests that negative regulatory element sequences are not primary targets of Nef regulation in stimulated T cells. An HIV-1-CAT plasmid containing mutated NF-κB sequences (23) was induced, at most, only twofold above basal levels, and induction was independent of cell type and Nef expression (Fig. 4a and b).

The importance of NF-κB with respect to the induction of IL-2 by T-cell mitogens was demonstrated by Hoyos et al. (17). These authors showed that the induction of CAT activity was prevented up to 80% with IL-2-CAT constructs bearing mutations in the NF-κB site compared with that of IL-2-CAT constructs containing wild-type NF-κB recognition sequences (17). As previously reported (21), we found that Nef profoundly suppressed the induction of CAT activity directed by the IL-2-CAT plasmid in the 133 cells (Fig. 4a). Whereas there was a 50- to 60-fold induction of CAT activity in the 22F6 cells, there was only a 2- to 3-fold induction in the 133 cells (Fig. 4a). Although NF-κB appears to play an important role in IL-2 induction, it is possible that Nef blocks other factors in addition to NF-κB which may be required for the efficient induction of IL-2 gene expression. This possibility may explain the dramatic suppressive effect of Nef on IL-2 induction compared with the results of Hoyos et al. (17). CAT activity generated by the IL-2-CAT construct was induced to a much lower extent in the Jurkat E6-1 cells. This result is likely due to differences that exist between Jurkat E6-1 and J25 cells. Despite the low level of induction of the IL-2 promoter in the Jurkat E6-1 cells, CAT activity was higher in the Jurkat/LfenSN cells than in the Jurkat/LnefSN cells (Fig. 4b). Nef did not affect CAT activity driven by the SV40 early promoter or the promoters from Rous sarcoma virus, cytomegalovirus, or Mason-Pfizer monkey virus, indicating that Nef specifically suppressed the HIV-1 and IL-2 promoters (data not shown). The Jurkat E6-1 cells were transfected with equivalent efficiency; however, the Nef-expressing 133 cells were more easily transfected than were the control cells (22F6 cells). Therefore, CAT activity generated by an RSV-CAT plasmid that was transfected in parallel was used to assess the transfection efficiency and to normalize the CAT activity derived from the HIV-1-CAT and IL-2-CAT constructs.

The observation that Nef prevents IL-2 induction (Fig. 4a) (21), coupled with the demonstrations that IL-2 induction requires CD4 and $p56^{lck}$ (11) and NF-κB recruitment (17), provides additional evidence to suggest that Nef uncouples signals originating from the TCR complex. Furthermore, the TCR complex induces NF-κB activity after treatment with antibodies to either CD2 or CD3 (5). Nef inhibits the induction of IL-2 by both of these stimuli (21).

Interestingly, Nef has been reported to down-modulate the surface expression of CD4 (10, 15). Although Nef did not affect the rate of CD4 transcription or translation (10), the mechanism by which Nef mediates the down-modulation of CD4 at the cell surface remains unclear. The connection between Nef-mediated negative effects on CD4 cell surface expression and HIV-1 and IL-2 regulation has not yet been established.

Previously, we and others reported that HIV-1 Nef mediated HIV-1 transcriptional suppression (1, 25). Some investigators were unable to confirm this effect (16, 19); however, differences in experimental approaches may explain the apparent discrepancy. For the first time, the data presented here suggest that the primary underlying event in Nef-mediated transcriptional repression in activated T cells is the inhibition of induction of NF-κB activity. In vivo, this suppression may limit the production and cell surface expression of viral gene products in infected cells, thereby allowing the cells to evade clearance by the cellular and humoral arms of the immune response. This model for Nef-mediated viral persistence in vivo may be consistent with the results of Kestler et al., which demonstrated that the presence of an intact *nef* gene was required to prolong simian immunodeficiency virus infection and induce pathogenesis in infected macaques (18). Furthermore, we and others demonstrated that simian immunodeficiency virus Nef inhibited simian immunodeficiency virus replication in vitro in a way that was analogous to the way in which HIV-1 Nef inhibited HIV-1 (4, 24). It is possible that high-level Nef expression early after infection (28) is sufficient to maintain HIV-1 in a relatively latent state, which may be critical for establishing a reservoir of HIV-1-infected cells and the eventual development of AIDS.

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