The human immunodeficiency virus (HIV) Nef protein plays a critical role in AIDS pathogenesis by enhancing replication and survival of the virus within infected cells and by facilitating its spread in vivo. Most of the data obtained so far have been in experiments with exogenous Nef protein, so far overlooking the effects of exogenous soluble Nef protein. We used recombinant exogenous Nef proteins to activate nuclear transcription factors NF-κB and AP-1 in the promonocytic cell line U937. Exogenous SIV and HIV-1 Nef proteins activated NF-κB and AP-1 in a dose- and time-dependent manner. Activation of NF-κB by exogenous Nef was concomitant to the degradation of the inhibitor of NF-κB, IκBα. In agreement with increased AP-1 activation, a time- and dose-dependent increase in JNK activation was observed following treatment of U937 cells with exogenous Nef. Since exogenous Nef activates the transcription factors NF-κB and AP-1, which bind to the HIV-1 long terminal repeat (LTR), we investigated the effect of exogenous Nef on HIV-1 replication. We observed that exogenous Nef stimulated HIV-1 LTR via NF-κB activation in U937 cells and enhanced viral replication in the chronically infected promonocytic cells U1. Therefore, our results suggest that exogenous Nef could fuel the progression of the disease via stimulation of HIV-1 provirus present in such cellular reservoirs as mononuclear phagocytes in HIV-infected patients.

Nef is a 27-kDa HIV1 protein that is produced early during infection and translated from multiply spliced viral mRNAs (1).

**References**

1. The abbreviations used are: HIV, human immunodeficiency virus; AP-1, activator protein-1; IκB, inhibitor of NF-κB; JNK, Jun N-terminal kinase; NF-κB, nuclear factor κB; NAK, Nef-associated kinase; PAK, p21-activated kinase; MAPK, mitogen-activated protein kinase; LTR, long terminal repeat; TNF, tumor necrosis factor; IL-1, IκBα kinase; MAPK, mitogen-activated protein kinase; SEAP, secreted alkaline phosphatase; LPS, lipopolysaccharide; FITC, fluorescein isothiocyanate; EMSA, electrophoretic mobility shift assay.
p65) is sequestered in the cytoplasm by interaction with a family of inhibitory proteins, or IκBα, including IκBα, IκBβ, IκBe, IκBγ, and the proto-oncogene Bel-3 (34). Following cell activation by a variety of extracellular stimuli, such as tumor necrosis factor α (TNF), IκBα is phosphorylated at the N-terminal residues Ser-32 and Ser-36 by the IκB kinase (IKK) complex, leading to ubiquitination and subsequent proteasome-mediated degradation, which allows NF-κB to translocate to the nucleus, where it activates gene expression.

Since the identification of NF-κB elements in the HIV LTR (32), multiple studies have assessed the effect of this family of transcription factors on the transcriptional regulation of the HIV LTR and its impact on HIV reactivation from latency (35–38). Study of the interaction between NF-κB and HIV in both human monocyteic cells and transformed human macrophages has mainly focused on how monocyte differentiation may lead to HIV expression (39, 40) and how HIV infection leads to NF-κB activation. In the promonocytic cell line U937, HIV activates the inducible pool of NF-κB as a result of enhanced IκBα degradation, which is believed to be secondary to IKK activation (41–44).

The activity of activator protein-1 (AP-1), a transcription factor, consisting of a homodimer and heterodimers of members of the Jun family (c-Jun, JunB, and JunD) and heterodimers of the Jun and Fos (c-Fos, FosB, Fra1, and Fra2) families, is regulated, at least in part, by the activation of c-Jun N-terminal kinase (JNK) (45). It has also been suggested that the activation of NF-κB is regulated by some upstream MAP kinases that also regulate JNK activation in the cells (46). Induction of AP-1 in macrophages by endogenous HIV-1 Nef has been reported to be a cell type-specific response that requires both Hck and MAPK signaling (47).

Exogenous Nef protein is detected in the serum of HIV-infected subjects (48). Both antibodies and cytotoxic T lymphocytes (CTLs) directed against Nef have been found in a large proportion of infected individuals (49, 50). This suggests that in vivo Nef is processed and presented by antigen-presenting cells, as the result of uptake of extracellular Nef possibly released by infected apoptotic cells (51). Exogenous Nef protein has been shown to enter the cell by adsorptive endocytosis following nonspecific binding to the surface of CD4+ T cells, primary macrophages, and U937 promonocytic cells (51) and to activate the signal transducer and activator of transcription 1 (STAT-1) in human monocyteic/macrophages (52). Confocal microscopy indicates that the intracellular distribution of internalized FITC-labeled recombinant Nef is identical to that of endogenously produced Nef, localizing both in an intracytoplasmic punctate pattern and at the cell margin (51, 53). Although most of the results reported so far in regard to signaling were following nonspecific binding to the surface of CD4+ T cells, primary macrophages, and U937 promonocytic cells (51) and to activate the signal transducer and activator of transcription 1 (STAT-1) in human monocyteic/macrophages (52), the DNA-protein complex formed was resolved from free oligonucleotide on a 6% native polyacrylamide gel. A double-stranded mutated oligonucleotide 5′-TTGGTACACTCCTTTCCGGGACCTCCTTCCAGGGAGGG-3′, which is used to examine the specificity of binding of NF-κB to the DNA. The specificity of the binding was examined by competition with unlabeled oligonucleotide, with a heterologous unlabeled oligonucleotide and with a consensus unlabeled oligonucleotide. The dried gels were visualized and radioactive bands quantified by a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) using ImageQuant software.

**Western Blot Analysis—**Cyttoplasmic extracts of U937 cells treated for various times with exogenous HIV protein were examined by Western blot protocol (Clontech, Palo Alto, CA). SEAP activity was assayed on a 96-well fluorescence plate reader (Fluoroscan II, Lab Systems) with excitation set at 360 nm and emission at 460 nm. This reporter system was specific since TNF-induced NF-κB SEAP activity was inhibited by overexpression of the IκBα mutant, IκBe-DN, which lacks Ser-32 and Ser-36 (60).

**P24 Assay—**U1 cells were treated with different concentrations of exogenous HIV-1 Nef or with TNF. At 48 h post-transfection, luciferase activity was measured in cell lysates using a luminometer (TD-20/20; Promega, Madison, WI) as previously reported (11). Values normalized to protein concentrations were expressed in fold increase over unstimulated control values.

**RESULTS**

**Exogenous Nef Activates NF-κB—**Treatment of U937 cells with exogenous SIV Nef for 90 min revealed a dose-dependent activation of NF-κB by EMSA (Fig. 1A), with maximum activation at ~50–100 ng/ml, but to a lesser extent than TNF, the most potent activator of NF-κB (3.5-fold versus 5.2-fold induction). Exogenous SIV Nef activated NF-κB in a time- and dose-dependent manner reaching a peak by 90 min (Fig. 1B). The gel shift band was specific as formation of the complex was blocked with an unlabeled oligonucleotide and was supershifted by...
either anti-p50 or anti-p65 antibody alone, and also by a mixture of anti-p50 and anti-p65 antibodies (Fig. 1C), indicating that it is composed of p50 and p65 subunits. To rule out the possibility that a TNF inducer, such as lipopolysaccharide (LPS), produced the activity, we tested recombinant HIV-1 Nef proteins derived from three HIV-1 isolates, NL4–3, SF2, and BH10, in regard to NF-κB activation mediated by exogenous Nef. We observed that exogenous SF2 Nef protein activated NF-κB in U937 cells treated with exogenous SIV Nef (50 ng/ml), indicating that exogenous Nef protein, but not LPS contamination, was responsible for NF-κB activation. The pretreatment of exogenous SIV Nef protein with a neutralizing anti-Nef monoclonal antibody blocked NF-κB activation (Fig. 1D), thereby indicating the effect was Nef-specific.

The degradation of IκBα in U937 cells treated with exogenous SIV Nef protein for different periods of time was also examined using Western blot analysis. We observed that IκBα started to degrade at 15 min, degraded maximally by 30–60 min, and started to be resynthesized at 90 min (Fig. 1E, upper panel). The presence of a slow migrating band of IκBα in samples prepared from SIV Nef-treated cells suggested the appearance of the phosphorylated form of IκBα, which is required for IκBα degradation. The induction of the phosphorylated form of IκBα by exogenous SIV Nef was detected by using an antibody directed against the phosphorylated form of IκBα, with a maximum detection by 30 min (Fig. 1E, lower panel).

Since SIV Nef and HIV-1 Nef may differ in regard to some of their biological functions (62), we tested recombinant HIV-1 Nef proteins derived from three HIV-1 isolates, NL4–3, SF2, and BH10, in regard to NF-κB activation in U937 cells. We observed that exogenous SF2 Nef protein activated NF-κB in a time-dependent manner (Fig. 2A). The gel-shift band was specific as formation of the complex was blocked with an unlabeled NF-κB oligonucleotide, but not with a mutated NF-κB oligonucleotide or with a heterologous oligonucleotide (Fig. 2B). The gel shift band was supershifted by either anti-p50 or anti-p65 antibody alone (Fig. 2C), indicating that it is composed of p50 and p65 subunits. Nef-induced NF-κB activation was also observed following treatment of U937 cells with exogenous HIV-1 BH10 and NL4–3 Nef proteins (Fig. 2D), indicating that NF-κB activation mediated by exogenous Nef is viral isolate-independent.

Exogenous Nef Activates AP-1 and JNK—Most agents that activate NF-κB also activate the transcription factor AP-1 (45),...
Therefore, we investigated the ability of exogenous SIV Nef protein to activate AP-1 in U937 cells. Exogenous SIV Nef protein activated AP-1 in a dose-dependent manner, but to a lesser extent than TNF (3.5-fold versus 5-fold induction) (Fig. 3A). AP-1 activation by exogenous HIV-1 Nef was time-dependent, with optimum activation occurring at ~90 min (Fig. 3B). Supershift analysis with specific antibodies against c-Fos and c-Jun indicated that AP-1 activation induced by exogenous HIV-1 Nef consisted of Fos and Jun (Fig. 3C). Lack of supershift by unrelated antibodies and disappearance of the AP-1 band by competition with unlabeled oligonucleotide indicate that the interaction was specific (Fig. 3C).

Activation of JNK is another early event initiated by many stress stimuli and is required for AP-1 activation (45). Treatment of U937 cells with exogenous SIV Nef protein led to an increase in JNK activity in a time- (Fig. 3D) and dose-dependent manner (Fig. 3E). The overall level of JNK activation triggered by exogenous SIV Nef protein was less than that observed following TNF treatment (3-fold versus 6-fold) (Fig. 3E).

Since SIV Nef and HIV-1 Nef could differ in regard to their biological functions (62), we tested recombinant HIV-1 Nef proteins derived from three HIV-1 isolates, NL4–3, BH10, and SF2 in regard to AP-1 activation in U937 cells. Exogenous BH10 Nef protein activated AP-1 in a time-dependent (Fig. 4A) and dose-dependent manner (data not shown). The gel shift band was supershifted by either anti-c-Fos or anti-c-Jun antibody alone, but also by a mixture of anti-c-Fos and anti-c-Jun antibodies (Fig. 4C), indicating that it is composed of c-Fos and c-Jun subunits.
AP-1 activation followed treatment of U937 cells with exogenous HIV-1 Nef proteins derived from NL4–3, BH10, and SF2 isolates (Fig. 4D), indicating that AP-1 activation mediated by exogenous HIV-1 Nef was viral isolate-independent.

Exogenous Nef Stimulates HIV-1 LTR—We assessed whether NF-κB activation triggers gene expression in U937 cells treated with exogenous Nef proteins. We examined the effect of exogenous SIV Nef on NF-κB-driven SEAP gene expression in U937 cells. SIV-Nef enhanced SEAP gene expression in a dose-dependent manner, comparable with TNF (Fig. 5A). SEAP gene expression was NF-κB-specific, since it was abolished by IκBa-DN, an IκBa mutant lacking Ser-32 and Ser-36 (Fig. 5A).

Then we assessed the effect of exogenous HIV-1 Nef on NF-κB-dependent LTR stimulation. U937 cells were transiently transfected with a target plasmid that contains the luciferase reporter gene under the control of the HIV-1 LTR promoter, pLTR-Luc (61). Twenty-four hours later, transfected cells were treated for 24 h with different concentrations of exogenous HIV-1 NL4–3 Nef and harvested, and luciferase activity was measured in cell lysates. Exogenous HIV-1 Nef stimulation doubled LTR activation over untreated control cells (Fig. 5B). As a positive control, TNF treatment of transfected U937 cells increased HIV-1 LTR stimulation by 2.5-fold (Fig. 5B). LTR activation induced by exogenous Nef was not observed when a plasmid containing a mutated NF-κB site, pLTR-mut-NFκB-Luc, was used instead of pLTR-Luc (Fig. 5B). These data indicate that exogenous HIV-1 Nef activated the LTR via NF-κB stimulation in promonocytic U937 cells.

Exogenous Nef Stimulates HIV-1 Replication in Chronically Infected Promonocytic U1 Cells—NF-κB and AP-1 DNA-binding sites are present in the HIV-1 LTR. Therefore, we determined the effect of exogenous Nef on provirus transcription in the promonocytic cell line U1, U937 cells that contain two integrated HIV copies per cell (57). We measured HIV-1 replication in U1 cells following treatment with exogenous HIV-1 Nef. Exogenous HIV-1 NL4–3 Nef stimulated viral replication in U1 cells as measured by p24 assay (Fig. 6A). To rule out the possibility that a TNF inducer, such as LPS, enhanced viral replication in U1 cells, we boiled exogenous Nef at 100 °C. Boiling abolished exogenous Nef-induced replication in U1 cells (Fig. 6A), indicating that Nef protein, but not LPS contamination, was responsible for enhanced viral replication. Exogenous HIV-1 NL4–3 Nef stimulated HIV-1 replication in a dose-dependent manner (Fig. 6B). The stimulation of HIV-1 replication by exogenous Nef was significantly diminished by a neutralizing anti-HIV-1 Nef antibody, and therefore was Nef-specific (Fig. 6B).

DISCUSSION

Exogenous Nef protein has been detected in serum from HIV-infected subjects (48). However its significance in regard to HIV pathogenesis has not been studied. Here we have demonstrated that exogenous Nef proteins derived from SIV and HIV-1 isolates activate NF-κB, AP-1, and JNK in promonocytic U937 cells. We observed that exogenous HIV-1 Nef stimulates HIV-1 LTR via NF-κB activation. We also found that exogenous Nef stimulates HIV-1 replication in chronically infected U1 promonocytic cells. These data indicate that exogenous Nef
enhances HIV-1 replication in latently infected promonocytic cells and could favor the spread of the disease via enhancement of viral replication from latently infected cellular reservoirs in HIV-infected subjects.

Increased NF-κB DNA binding has been described in promonocytic cell lines infected with HIV (41). Here we report that exogenous Nef activates NF-κB and induces IKKβ phosphorylation and degradation in promonocytic U937 cells. Since phosphorylation of IkBα at serine is induced, exogenous Nef probably activates IKK-β, the only kinase known to phosphorylate IkBα directly (63). IKK-β is regulated by several upstream kinases and endogenous Nef has been reported to bind directly to such members of the Src kinase family as Hck, Lyn, Lck, and Fyn (17, 22, 26, 27, 64). Although Lck has been implicated in NF-κB activation (60, 65), it is not expressed in primary macrophages and in U937 cells (66). In agreement with this observation we observed NF-κB activation in Lck-deficient cells treated with exogenous Nef (data not shown).

Our data also show that exogenous Nef activates AP-1 and JNK in promonocytic cells, indicating that exogenous Nef interferes with the MAPK pathway. Endogenous Nef has been reported to directly interact with Lck and MAPK inhibiting their kinase activity in T cells (23). These results indicate that exogenous and endogenous Nef might have different effects on the MAPK pathway.

Activation of transcription factors NF-κB and AP-1 was observed after treatment with exogenous Nef derived from SIV and HIV-1 isolates. These results could indicate that conserved regions between SIV and HIV-1 Nef proteins are involved in this phenomenon. In fact, SIV Nef is larger than HIV-1 Nef, and the molecules share 38% amino acid homology (62). The most homologous regions are the N-terminal myristylation region and a highly conserved core region (62). Additional studies are required to determine the regions of SIV and HIV-1 Nef involved in NF-κB and AP-1 activation in promonocytic cells. The amounts of exogenous Nef detected in the serum of

FIG. 4. AP-1 activation in U937 cells treated by exogenous HIV-1 Nef. A, time course-dependent responses of AP-1 activation induced by exogenous HIV-1 Nef in U937 cells. U937 cells (1.5 × 10⁶/ml) were treated with 1000 ng/ml exogenous HIV-1 BH10 Nef for different periods of time, and then AP-1 activation was measured by EMSA, as described under “Experimental Procedures.” B, specificity of AP-1 activation by exogenous HIV-1 Nef. Nuclear extracts from U937 cells treated with 1000 ng/ml exogenous HIV-1 BH10 Nef were incubated for 20 min with increasing concentrations of unlabeled homologous or consensual AP-1 oligonucleotides, and then assayed for AP-1 activation. Untreated and treated U937 cells were negative and positive controls, respectively. C, supershift of AP-1 activation by exogenous HIV-1 Nef. Nuclear extracts from U937 cells treated with exogenous HIV-1 BH10 Nef were incubated for 30 min with anti-c-Fos, anti-c-Jun, or anti-c-Fos + anti-c-Jun, and then assayed for AP-1 DNA binding activity. Untreated U937 cells, used as a negative control, are also represented. D, AP-1 activation in U937 cells treated by exogenous Nef derived from different HIV-1 isolates. Following treatment of U937 cells with exogenous HIV-1 Nef derived from NL4–3, BH10, and SF2 isolates (1000 ng/ml), AP-1 binding was detected by EMSA at the indicated times post-treatment and measured as fold induction versus untreated cells, using a PhosphorImager.
HIV-infected subjects is around 10 ng/ml (48). At 10 ng/ml, we
observed that recombinant Nef protein activates NF-κB, AP-1,
and JNK (Figs. 1 and 3). In agreement with our results, it has
been reported that recombinant Nef protein activates STAT1 in
monocyte-derived macrophages starting at a concentration of 10
ng/ml (52), pointing out the possibility that our observations
reflect phenomena actually occurring in vivo. Also, we cannot
rule out that higher amount of exogenous Nef, e.g. 100 ng/ml,
could be present in tissue compartments such as lymph nodes
where macrophages and infected lymphocytes tightly interact
(67, 68). The low amounts of exogenous Nef detected in the serum
of HIV-infected subjects could also result from the presence of
immune complexes Nef-antiNef as reported previously (48).

The features observed in promonocytic cells and primary
macrophages following exposure to exogenous Nef are very
similar to those observed following TNF treatment. Both exog-
igenous Nef and TNF activate NF-κB, AP-1, and JNK, suggesting
that they might modulate the cellular machinery in a similar
way and therefore might have the same effect on HIV replica-
tion in mononuclear phagocytes. We observed that exogenous
HIV Nef, like TNF treatment (69), stimulates HIV-1 replication
in the chronically infected promonocytic cell line U1.

Although exogenous Nef binds to the surface of macro-
phages, the molecular mechanisms underlying this interaction
are not yet unveiled (70). Exogenous Nef enters the cell by
adsorptive endocytosis following nonspecific binding to the sur-
face of U937 cells (51, 70). No specific receptor for exogenous
Nef protein has been described (51). However, we cannot ex-
clude possible low-level expression of a potential cell surface
Nef receptor that could not be revealed by fluorescence-acti-
vated cell sorting (FACS) analysis on rNef-FITC-treated cells.

Through cytofluorometric analyses, the internalization of
FITC-conjugated recombinant Nef into primary macrophages

Fig. 5. Exogenous SIV Nef and HIV Nef activate NF-κB-driven
reporter gene expression. A, U937 cells were transiently transfect-
ected with the SEAP expression plasmid for 10 h before treatment with
increasing concentrations of exogenous SIV Nef and TNF. After 24 h,
cell culture-conditioned medium was harvested and analyzed for alka-
line phosphatase activity as described under “Experimental Proce-
dures.” The specificity of the reporter system was confirmed using an
IκBα mutant IκBa-DN lacking Ser-32 and Ser-36 residues. B, exoge-
 nous HIV-1 Nef activates HIV-1 LTR via NF-κB stimulation. U937 cells
were transiently transfected with 750 ng of p-LTR-Luc or with 750 ng
of p-LTR-mut-NF-κB-Luc. Twenty-four hours later, transfected cells
were mock-treated or treated with increasing concentrations of exoge-
 nous HIV-1 NL4–3 Nef or TNF (100 pm), and luciferase activity was
measured in cell lysates. The mock-treated value of the wild-type LTR
reporter construct and of the mutant-LTR reporter construct were
arbitrarily set at a value of 1. Values represent the means of duplicate
samples. A representative experiment of two independent transfections
is shown.

Fig. 6. Exogenous HIV-1 Nef protein stimulates HIV-1 replica-
tion in chronically infected U1 promonocytic cells. A, time course
of HIV-1 replication in U1 cells treated with exogenous HIV-1 Nef. U1
cells were treated with 1000 ng/ml exogenous HIV-1 NL4–3 Nef or with
1000 ng/ml exogenous HIV-1 NL4–3 Nef boiled at 100 °C for 20 min,
and p24 was measured at different times post-treatment in culture
supernatants as reported under “Experimental Procedures.” Untreated
U1 cells were used as a negative control (p24 < 50 pg/ml; data not
shown). B, dose response and specificity of viral replication induced in
U1 cells by exogenous HIV-1 NL4–3 Nef. U1 cells were treated with
increasing concentrations of exogenous NL4–3 HIV-1 Nef (0, 100, 1000
ng/ml) in the absence or presence of neutralizing anti-Nef antibody at
10 μg/ml, and p24 was measured in culture supernatants at day 7
post-treatment. Results are presented as a histogram. For each point,
p24 was quantified from independent duplicates, and the means of the
duplicate samples are presented. A representative experiment of two
independent p24 assays is shown.
was observed (51). Confocal microscopy indicated that the intracellular distribution of internalized recombinant Nef was identical to that of endogenously produced Nef, localizing both in an intracytoplasmic punctate pattern and at the cell margin (51, 53). The identical intracellular distribution of internalized recombinant Nef and of endogenously produced Nef could explain why exogenous Nef protein activates NF-κB, AP-1, and JNK and stimulates the HIV-1 LTR. Exogenous Nef might interfere with intracellular signaling pathways downstream of TNF receptors and thereby could mimic the effect of TNF on primary macrophages. Exogenous Tat has been shown to activate NF-κB, AP-1, and JNK and to trigger the release of proinflammatory cytokines from primary macrophages including TNF, IL-1β, IL-6, and IL-8 (71, 72). Several features indicate that the activation of NF-κB, AP-1, and JNK by exogenous Nef is a direct intrinsic effect of the Nef protein and is not mediated via the release of endogenous TNF. We did not detect increased TNF levels in culture supernatants of U937 cells treated with exogenous Nef (data not shown). The time curve of NF-κB, AP-1, and JNK activation is similar following treatment with either exogenous Nef or TNF, with DNA binding starting at 30 min post-treatment and with a peak at 120 min post-treatment (73). Our results suggest a redundancy between Nef and TNF in regard to the activation of NF-κB, AP-1, and JNK in promonocytic cells. Thus, exogenous Nef mimics TNF biological effects, suggesting that a viral protein could fuel the progression of the disease even in the absence of proinflammatory cytokines. This might be of critical importance at early stages of the disease when chronic immune activation is not yet predominant and viral factors are needed to establish a productive infection.

Our results indicate that exogenous Nef protein and exogenous Tat protein might share similar biological functions. Thus, like exogenous Nef protein, exogenous Tat protein activates NF-κB, AP-1, and JNK, transactivates the HIV-1 LTR when added exogenously to U937 cells growing in culture and stimulates HIV-1 replication in U1 cells (73–77). Also, like exogenous Nef protein, exogenous Tat protein is detected in the serum of HIV-infected subjects (78) and can be released from HIV-infected cells (79). Similarly, exogenous Tat protein has been reported to bind nonspecifically to the cell surface and to enter U937 promonocytic cells by adsorptive endocytosis in vitro (74, 80) and in vivo (81), contributing to the transcellular activation of HIV-1 LTR promoter in latently infected cells (82, 83). Also, fluorescent experiments with rhodamine-conjugated Tat showed punctate staining on the cell surface and then localization to the cytoplasm and nucleus (74). Thus, both exogenous Nef protein and exogenous Tat protein can enter target cells and activate HIV-1 LTR. Similar effects of Nef protein and Tat protein on T cell apoptosis have been reported. Both endogenous Tat and endogenous Nef have been shown to block apoptosis in HIV-infected T cells, while both exogenous Tat and exogenous Nef have been shown to deliver proapoptotic signals to uninfected T cells (10, 12, 29, 84). All together these data indicate a redundancy between the effects of exogenous Nef and exogenous Tat on several biological functions including transcription activation.

Since exogenous Nef activates AP-1, NF-κB, and JNK, but to a lesser extent than TNF, exogenous Nef may play a role in macrophage activation observed during HIV pathogenesis. Nef protein is detected in the serum of HIV-1 infected patients (48), indicating that exogenous Nef could participate in the reactivation of the virus from latency, especially in mononuclear phagocytes. Although endogenous Nef prevents T cell apoptosis in HIV-infected cells (10–12), the release of soluble Nef from the infected cells triggers the apoptosis of uninfected CD4+ T cells present in the vicinity (70). Increased T cell apoptosis has been reported following treatment with exogenous Nef (70), and we observed that exogenous Nef triggers T cell apoptosis via activation in poly(ADP-ribose) polymerase (PARP). Thus, exogenous Nef protein could participate to the immune suppression observed in HIV-infected subjects via T cell depletion of uninfected bystander cells. Therefore, exogenous Nef protein could favor the progression of the disease via both increased HIV-1 replication from latently infected mononuclear cells and enhanced immune suppression due to T cell apoptosis.

In conclusion, our results show that exogenous Nef activates NF-κB, AP-1, and JNK, and stimulates viral replication in the chronically infected promonocytic cells U1 via activation of the HIV LTR. This observation suggests a critical role for exogenous Nef in AIDS pathogenesis via enhancement of HIV-1 replication from latently infected mononuclear phagocytes. A better understanding of the mechanisms underlying the replication of HIV-1 from latently infected cellular reservoirs is likely to lead to new therapeutic approaches, which could help to clear the reservoirs of virions in HIV-infected individuals.

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Exogenous Nef Protein Activates NF-κB, AP-1, and c-Jun N-Terminal Kinase and Stimulates HIV Transcription in Promonocytic Cells: ROLE IN AIDS PATHOGENESIS
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