HIV-1 Nef Inhibits Lipopolysaccharide-induced IL-12p40 Expression by Inhibiting JNK-activated NFκB in Human Monocytic Cells*

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Impaired cellular immunity caused by decreased production of Th1-type cytokines, including interleukin-12 (IL-12) is a major feature of HIV-1-associated immunodeficiency and acquired immunodeficiency syndrome. IL-12p40, an inducible subunit shared between IL-12 and IL-23, plays a critical role in the development of cellular immunity, and its production is significantly decreased during HIV infection. The mechanism by which HIV induces loss of IL-12p40 production remains poorly understood. We have previously shown that lipopolysaccharide (LPS)-induced IL-12p40 production in monocytic cells is regulated by NFκB and AP-1 transcription factors through the activation of two distinct upstream signaling pathways, namely c-Jun-N-terminal kinase (JNK) and the calmodulin-dependent protein kinase-II-activated pathways. Herein, we show that intracellular nef expressed through transduction of primary monocytes and promonocytic THP-1 cells with retroviral-mediated nef gene inhibited LPS-activated IL-12p40 production by inhibiting the JNK mitogen-activated protein kinases without affecting the calmodulin-dependent pathway. In addition, our results indicate for the first time that intracellular nef inhibited IL-12p40 transcription, which may cause impairment of HIV-specific cellular immunity by inhibiting the production of Th1-type cytokines such as IL-12 (1–5). IL-12 acts as a bridge between innate and adaptive immune responses and plays a critical role in the immunopathogenesis of various diseases, including HIV infection, inflammation, and autoimmune disorders (3, 4, 6, 7). It promotes Th1-type cell-mediated immune responses by inducing cytokines from NK and T cells and enhances their IFN-γ production (8–10). It is a 70-kDa heterodimer composed of p35 and p40 subunits that are disulfide-linked to form biologically active IL-12 (9, 10). The p35 subunit is inducible and differentially regulated at transcription levels following cells, whereas the p40 subunit is constitutively expressed in various cell types (9, 11). The p35-p40 heterodimer or IL-12 proliferation is regulated by another transcription factor, interferon-γ-responsive elements (IFN-γRE) (6).

HIV5 infection results in a progressive loss of general and specific immune responses by inducing interferon-γ and TNF (12). Multiple transcription factors, including NFκB and AP-1, have been demonstrated to regulate IL-12p40 transcription in LPS-stimulated human monocytic cells (7, 11–14). We and others have shown that c-Jun-N-terminal kinase (JNK) plays a key role in the regulation of IL-12p40 production in LPS-stimulated human and monocytic cells (7, 11–14). Recently, we have also shown that LPS-induced IL-12p40 production is regulated by another distinct pathway, the calmodulin/CaM-activated protein kinase (CaMK-II)–activated phosphatidylinositol-3 kinase pathway (16). Interestingly, both pathways regulated IL-12p40 production through the NFκB and AP-1 transcription factors (13, 16).

The abbreviations used are: HIV, human immunodeficiency virus; IL-12, interleukin-12; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; ERK, extracellular signal-regulated kinase; EUSA, enzyme-linked immunosorbent assay; RT, reverse transcriptase; CaM, calmodulin; CaMK-II, calmodulin-dependent kinase-II; JNK, c-Jun-N-terminal kinase; MAPK, mitogen-activated protein kinase; pSRx, the retrovirus containing the empty vector; pSRx-Nef, pSRx containing the Nef gene; SEK1, stress-activated protein kinase/extracellular signal-regulated kinase 1.
cells (5). We and others have shown that IL-12 production is decreased in HIV-infected patients and treatment with antiretroviral therapy enhanced IL-12 production (3–5, 18–22). Furthermore, exogenous addition of IL-12 enhanced IL-2 production, cell proliferation, and the development of cell-mediated cytotoxicity of HIV antigen-stimulated PBMCs from HIV-infected individuals (3, 4, 18, 19, 22–24).

To understand the mechanism underlying the loss of cell-mediated immune response during HIV infection and development of AIDS, it is imperative to investigate the signaling pathways responsible for the loss of Th1 cytokines IL-12 and IL-23 and in particular the inducible IL-12p40 subunit shared between these two cytokines. At present, little is known regarding the regulation and expression of IL-12p40 in monocytic cells following HIV infection. There is evidence to suggest that HIV regulatory protein nef, inhibits IL-12 synthesis. IL-12p40 production was shown to be suppressed in lymph nodes of macaques infected with simian immunodeficiency virus compared with those infected with the non-pathogenic nef-deleted strain, SIVmac239 (25). However, the exact role of nef and the mechanism involved in the inhibition of IL-12p40 production in monocytic cells are not known. Nef is a 27-kDa myristoylated protein expressed early in HIV infection (26). In addition to the well known down-regulation of the cell surface CD4 and MHC-I receptors (27, 28), nef uniquely can interact with a number of signaling molecules through its myristoylated protein expressed early in HIV infection (29–34). In this study, we show for the first time that intracellular expression of HIV-nef by retroviral transfection of THP-1 cells inhibited LPS-induced IL-12p40 by 95% (29–34). This led us to determine the mechanism by which nef inhibited IL-12p40 production in monocytic cells and promonocytes without affecting the activity of AP-1 transcription factor.

**MATERIALS AND METHODS**

**Cell Lines, Cell Culture, and Retroviral Packaging**—THP-1, a promonocytic cell line and 293T cells, the embryonic kidney epithelial cells, were obtained from the American Type Culture Collection (Manassas, VA). A retroviral packaging cell line, PT67, derived from NIH 3T3 cells and packaging virus with a polytropic envelope, was purchased from BD Biosciences Clontech (Mississauga, Ontario, Canada). Cells were cultured in Iscove’s modified Dulbecco’s medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Invitrogen), 100 units/ml penicillin, 100 µg/ml gentamicin, 10 mM HEPES, and 2 mM glutamine. The anti-mitogen-activated protein kinase (MAPKs), including extracellular signal regulated kinase (ERK1/2), p38, and JNK and anti-phospho MAPKs antibodies (Cell Signaling), anti-CaMK-II and phospho-CaMK-II antibodies (StressGen Biotechnologies, Victoria, British Columbia, Canada), the JNK inhibitor SP600125 (Biomol, Plymouth Meeting, PA), the calcium chelating agent EGTA (Sigma), SKF-96365 hydrochloride, an inhibitor of receptor-mediated calcium entry, W7 hydrochloride, a calmodulin antagonist, and KN-93, an inhibitor of CaMK-II (all from Calbiochem), were purchased.

**Isolation of Monocytes**—Monocytes were purified from PBMCs by negative selection as described earlier (16, 35). Briefly, PBMCs were incubated with magnetic polystyrene Dynabeads (Dynal Biotech, Oslo, Norway) coated with anti-CD2 (T cells) and anti-CD19 (B cells) antibodies for 30 min on ice for depletion of T and B cells, respectively. Cells were further incubated for 2 h at 37 °C to eliminate nonadherent cells. The monocytes obtained contained <1% T and B cells as determined by flow cytometry. Some experiments were confirmed by using monocytes isolated by automacs negative selection (Miltenyi Biotech Inc., Auburn, CA). Briefly, PBMCs were washed twice in phosphate-buffered saline containing 2% EDTA followed by incubation with automacs FcR blocking reagent along with biotin antibody mixture for 10 min at 4 °C. Following incubation, cells were treated with anti-biotin microbeads for 15 min at 4 °C. Cells were then washed once and subjected to automacs positive selection separation as per the manufacturer’s instructions. The monocyte populations thus obtained are >95% pure monocytes.

**Production of HIV-nef Retrovirus**—A retroviral vector pSRα-M viruses (pSRα-Nef) derived from HIV-1pNL4–3 (GenBankTM accession number K02007) was a gift from Dr. T. Smithgall (University of Pittsburgh) (33). The control retroviruses were produced in a retroviral packaging cell line, PT67 (Manassas, VA). A retroviral packaging cell line, PT67, was transduced with 4 µg of nef gene (pSRα-Nef) derived from HIV-1pNL4–3 (GenBankTM accession number K02007) HIV molecular clones were provided by Dr E. Cohen, University of Montreal. 293T cells were transfected with HIV-1pNL4–3 or Δnef-HIV-1pNL4–3 plasmids for 2 days using FuGENE6 as transfecting reagent as described earlier (13, 16).

**Infection of Monocytic Cells with nef Retrovirus and Measurement of IL-12p40 by ELISA**—Briefly, cells were cultured in 3 ml of virus-containing supernatant collected from packaging PT67 cells for 24 h and infected a second time under identical conditions for another 24 h followed by stimulation with LPS for various times. Cells were harvested for protein and RNA extraction. The supernatants were collected for measurement of IL-12p40 production by ELISA (R & D Systems) as described earlier (16, 35).

**Infection of Monocytic Cells with Wild-type and nef-deleted HIV Mutants**—Wild-type (HIV-1pNL4–3) and nef-deleted (Δnef-HIV-1pNL4–3) HIV molecular clones were provided by Dr E. Cohen, University of Montreal. 293T cells were transfected with HIV-1pNL4–3 or Δnef-HIV-1pNL4–3 plasmids for 2 days using FuGENE6 as transfecting reagent as described earlier (13, 16).

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Transfected with IL-12p40 Promoter-luciferase Reporter and Luciferase Assay—A series of truncated hIL-12p40 promoter fragments (−880 to +108) were generated by PCR and subcloned into the NheI/NcoI sites of pGL3B luciferase reporter plasmid as described earlier (13, 16). To generate mutations in key transcription factor binding sites, site-directed mutagenesis was performed by PCR using mutagenic primers. All the sequences were confirmed by the Biotechnology Research Institute, University of Ottawa. THP-1 cells were transiently transfected by FuGENE6 reagent (Roche Diagnostics) as described earlier (13, 16). Briefly, THP-1 cells infected with pSRα-Nef retroviruses were transfected with 1 μg of IL-12p40 promoter/luciferase constructs and 0.5 μg of the pSV-β-galactosidase (Promega, Madison, WI) by employing FuGENE6 reagent as described earlier (13, 16). After 24 h, cells were stimulated with LPS for another 24 h following which cells were assayed for luciferase and β-galactosidase activity by using respective assay kits (Promega). IL-12p40 promoter luciferase activity was normalized according to β-galactosidase values.

Electrophoretic Mobility Shift—Electrophoretic mobility shift assay was performed as described earlier (13, 16). Briefly, THP-1 cells infected with pSRα-Nef for 24 h each and stimulated with LPS for 2 h. The nuclear proteins were isolated by homogenization and subjected to nondenaturing PAGE. DNA-protein complexes were separated from unbound nucleotides by nondenaturing PAGE. The DNA probe was a 200-base pair fragment containing the B and AP-1 binding sites of the IL-12p40 promoter.

Intracellular nef Inhibits LPS-induced IL-12p40 Expression in Human Monocytic Cells—To determine the effect of intracellular HIV-nef on IL-12p40 expression in monocytic cells, HIV-nef retroviruses were generated to infect monocytic cells because of their ability to transduce gene with high efficiency. HIV-nef retroviruses were generated by transfecting amphotrophic packaging cell line PT67 with either pSRα-Nef retrovirus (pSRα-Nef) or the retrovirus containing the empty vector pSRα. The integration of pSRα-Nef in PT67 cells was verified by subjecting the genomic DNA to PCR and intracellular expression of nef protein was confirmed by Western blotting (Fig. 1, A and B). The supernatant from a 3-day culture of PT67 cells stably transfected with pSRα-Nef was used as a source of nef-containing retroviruses. The supernatants were assayed for determination of viral titers by infecting NIH 3T3 cells. Monocytes and THP-1 cells were infected with either pSRα-Nef or pSRα at a multiplicity of infection of 1 for 24 h. To obtain maximal viral infectivity, cells were infected again for another 24 h. THP-1 cells and monocytes infected with pSRα-Nef expressed nef as determined by PCR and Western blotting (Fig. 1, A and B).
Intracellular nef inhibits LPS-induced IL-12p40 expression in pSRα-Nef-transfected PT67 cells, and pSRα-Nef-retroviruses were infected with equal amounts of pSRα-Nef or control virus at a multiplicity of infection of 1 at 37 °C for 24 h. THP-1 cells stably transfected with pcDNA-Nef or control plasmid were infected with LPS for 24 h.

The expression of nef in THP-1 cells stably transfected with pcDNA-Nef was confirmed by stable transfection of THP-1 cells with a nef plasmid from another HIV strain (Fig. 1A, middle panel). Total RNA prepared from LPS-stimulated and pSRα-Nef- or pSRα-infected monocytes and THP-1 cells was analyzed for IL-12p40 mRNA expression by semi-quantitative RT-PCR. LPS-induced IL-12p40 mRNA production in monocytes and THP-1 cells was analyzed for IL-12p40 mRNA expression by semi-quantitative RT-PCR. LPS-induced IL-12p40 mRNA was determined by performing RT-PCR analysis for the neomycin gene present in both nef and pSRα vectors (Fig. 1A, lower panel).

The expression of nef in THP-1 cells stably transfected with pcDNA-Nef was confirmed by stable transfection of THP-1 cells with a nef plasmid from another HIV strain (Fig. 1A, middle panel). Total RNA prepared from LPS-stimulated and pSRα-Nef- or pSRα-infected monocytes and THP-1 cells was analyzed for IL-12p40 mRNA expression by semi-quantitative RT-PCR. LPS-induced IL-12p40 mRNA production in monocytes and THP-1 cells was analyzed for IL-12p40 mRNA expression by semi-quantitative RT-PCR. LPS-induced IL-12p40 mRNA was determined by performing RT-PCR analysis for the neomycin gene present in both nef and pSRα vectors (Fig. 1A, lower panel).

Inhibition of LPS-induced IL-12p40 expression by HIV- nef in monocytic cells was confirmed by stable transfection of THP-1 cells with a nef plasmid from another HIV strain (pcDNA-Nef). The expression of nef in THP-1 cells stably transfected with pcDNA-Nef was confirmed by PCR as well as immunoblotting (Fig. 1, A and B, right panels). To determine the effect of intracellular nef on IL-12p40 production, THP-1 cells stably transfected with pcDNA-Nef were analyzed for IL-12p40 mRNA by semi-quantitative RT-PCR. LPS-induced IL-12p40 mRNA expression was significantly inhibited in pcDNA-Nef-transfected THP-1 cells compared with the control plasmid (p < 0.001) as determined by ELISA and semi-quantitative RT-PCR analysis (Fig. 1, C and D, right panels).
The inhibitory effect of nef on IL-12p40 expression was also confirmed by infecting THP-1 cells with equal amounts (100 pg/ml p24) of nef-deleted (Δnef) HIV-1pNL4–3 and wild-type (HIV-1pNL4–3) HIV molecular clones. Two days after infection, cells were stimulated with LPS for another 24 h followed by measurement of IL-12p40 production by ELISA. Infection with wild type HIV significantly inhibited IL-12p40 production compared with the cells infected with Δnef-HIV-1pNL4–3 viruses (Fig. 1C, right panel).

Intracellular nef Down-regulates LPS-induced IL-12p40 Production by Selectively Inhibiting JNK MAPK—We have previously shown that LPS-induced IL-12p40 production in human monocytic cells is regulated by two distinct signaling pathways, namely the JNK MAPK and the CaMK-II pathways (13, 16). HIV-1-nef is also known to interfere with a number of signaling molecules, including MAPKs (30–32). It is possible that nef inhibits LPS-induced IL-12p40 expression through the inhibition of either JNK or CaMK-II alone or JNK and CaMK-II together. Therefore, to ensure that the activities of JNK and Ca2+/CaM/CaMK-II are not influenced by retroviruses, we first determined that LPS-induced IL-12p40 expression in control pSRα-infected monocytes and THP-1 cells is regulated by JNK and CaMK-II (Fig. 2A, left panel). LPS-induced IL-12p40 expression is regulated by JNK and CaMK-II in control pSRα-infected THP-1 cells and monocytes by employing specific pharmacological inhibitors. Prior to LPS stimulation, cells were treated for 2 h with various concentrations of either JNK inhibitor SP600125 (39), calcium chelating agent EGTA, SKF96365, W-7, and KN-93 at indicated concentrations for 2 h followed by stimulation with LPS (1 μg/ml) for another 24 h. The supernatants were analyzed for IL-12p40 production by ELISA. The results shown are mean ± S.D. of four independent experiments.

FIGURE 2. LPS-induced IL-12p40 production is regulated by the JNK and the CaM/CaMK-II activated pathways in pSRα-infected monocytes and THP-1 cells. Monocytes (left panel) and THP-1 cells (right panel) were infected twice with control pSRα retroviruses for 24 h each. Cells were treated with (A) JNK inhibitor SP600125 or (B) various inhibitors specific for calcium signaling (EGTA, SKF96365, W-7, and KN-93) at indicated concentrations for 2 h followed by stimulation with LPS (1 μg/ml) for another 24 h. The supernatants were analyzed for IL-12p40 production by ELISA. The results shown are mean ± S.D. of four independent experiments.

To determine if HIV-nef affects LPS-activated JNK and/or calcium signaling, monocytes and THP-1 cells were infected with either pSRα-Nef or pSRα retroviruses followed by LPS stimulation for 0–120 min. The pSRα-Nef-infected THP-1 cells and monocytes failed to significantly induce JNK phosphorylation at any time post-stimulation (Fig. 3A). The ERK and p38 MAPKs have also been shown to regulate IL-12p40 production in some cell types (42, 43). Therefore, we determined if nef interfered with p38 and ERK activation following LPS stimulation. However, following stripping of the same blots, LPS induced similar levels of ERK (Fig. 3B) and p38

FIGURE 3. Nef inhibits LPS-induced phosphorylation of JNK, but not ERK MAPKs. 106 THP-1 cells (left panel) and monocytes (right panel) were infected twice with either pSRα or pSRα-Nef retroviruses followed by LPS stimulation (1 μg/ml) for the indicated times. Total proteins were analyzed for JNK and ERK phosphorylation by Western blot analysis using anti-phospho-JNK and anti-phospho-ERK1/2 antibodies, respectively. To ensure equal protein loading, the blots were stripped and reprobed with anti-JNK and anti-ERK1/2 antibodies, respectively. Quantitation and normalization as percentage units to the kinase content are shown in the bottom panels. Results shown are a representative of three independent experiments.
Regulation of Human IL-12p40 by HIV-nef

Intracellular nef-mediated Inhibition of the LPS-activated JNK—Because LPS-induced IL-12p40 is regulated by NFκB and AP-1 transcription factors (sequence shown in Fig. 6A) in human monocytes (13, 16), we determined if intracellular nef inhibits LPS-induced IL-12p40 transcription by inhibiting the promoter activity of NFκB, AP-1 or both transcription factors. To confirm our earlier observations and to ensure that infection with control retroviruses did not impair IL-12p40 promoter activity, THP-1 cells infected with the pSRα retroviruses were transfected with a series of IL-12p40 promoter 5′-deletion mutants linked with the luciferase reporter plasmid, pGL3B, for 24 h followed by stimulation with LPS. Subsequent analysis of luciferase activity revealed that transfection with the full-length −880 to +118 promoter construct induced significant luciferase activity compared with the cells transfected with the control vector (Fig. 6B). In contrast, transfection with the vectors containing mutant NFκB in the presence of the PU.1 binding sequence (−128, pIL-12p40.PU.1, and NFκBm), abrogated luciferase activity (Fig. 7B).
Regulation of Human IL-12p40 by HIV-nef

To investigate if intracellular nef inhibited LPS-induced IL-12p40 transcription by inhibiting NFκB and/or AP-1 activity, THP-1 cells infected with pSRα-Nef were transiently cotransfected with either the full-length IL-12p40 promoter construct (−880/+118 bp), NFκB containing plasmid (−116, pIL-12p40.NFκB) or the vectors containing wild-type AP-1 and mutant NFκB sites (−232, pIL-12p40.AP-1, Ets-2, PU.1, and NFκBm). LPS-induced luciferase activity was significantly reduced in nef-infected cells transfected with full-length IL-12p40 promoter construct and NFκB containing −116, pIL-12p40.NFκB plasmids compared with the cells infected with control virus (Fig. 6C). LPS stimulation induced a significant −3-fold increase in luciferase activity in pSRα-infected cells transfected with a plasmid containing wild-type AP-1 and mutant NFκB sites (−232, pIL-12p40.AP-1, Ets-2, PU.1, and NFκBm), although this increase in luciferase activity was low compared with the cells transfected with plasmids containing wild-type NFκB sites. On the other hand, LPS-induced luciferase activity was not significantly reduced in pSRα-Nef-infected cells transfected with the cells containing wild-type AP-1 and mutant NFκB sites with the cells infected with the control virus. Similar results were observed when cells transfected with the pcDNA-Nef expression vector used for the selected promoter constructs (data not shown). These results suggest that intracellular nef inhibited IL-12p40 transcription primarily by inhibiting NFκB activity.
observed in AIDS patients (1, 3, 4). However, the mechanism by which HIV-1 infection inhibits IL-12 production remains unknown. Herein, we show for the first time that intracellularly expressed nef in primary monocytes and THP-1 cells resulted in the inhibition of LPS-induced IL-12p40 expression. Moreover, intracellular nef inhibits IL-12p40 expression through the selective inhibition of JNK-activated NFκB pathway in these cells.

HIV regulatory proteins tat, nef, and vpr are known to modulate production of cytokines, including IL-12 (25, 45–47). Variable effects of tat and nef on IL-12 expression in B cells and monocytes have been observed possibly due to different cell types and the models used to deliver these genes/gene products into the cells. For example, tat was shown to suppress IL-12 production in human PBMCs and enhance its production in dendritic cells (45, 48). We have previously shown that tat did not affect IL-12p40 production in either unstimulated or LPS-stimulated monocytic cells (25, 49 and data not shown). On the other hand, HIV vpr was shown to inhibit IL-12 production by down-regulating IL-12p35 subunit without affecting the synthesis of IL-12p40 (47). Similarly, there are reports showing that recombinant nef in IL-12p40 production was suppressed in THP-1 cells with simian virus 40 (SV40) large T antigen (29). Intracellular nef in infected cells (25).

For example, tat was shown to suppress IL-12 production in human PBMCs and enhance its production in dendritic cells (45, 48). We have previously shown that tat did not affect IL-12p40 production in either unstimulated or LPS-stimulated monocytic cells (25, 49 and data not shown). On the other hand, HIV vpr was shown to inhibit IL-12 production by down-regulating IL-12p35 subunit without affecting the synthesis of IL-12p40 (47). Similarly, there are reports showing that recombinant nef in IL-12p40 production was suppressed in THP-1 cells with simian virus 40 (SV40) large T antigen (29).

In contrast, HIV-nef was shown to inhibit macrophage colony stimulating factor receptor signaling through Hck activation in monocytes/macrophages (54). Our results suggest that intracellular nef interferes with the LPS-activated JNK pathway to inhibit IL-12p40 production. LPS mediates its effects through the CD14/TLR4 receptor complex involving the activation of tyrosine and serine/threonine kinases, including protein kinase C, MAPKs, phosphatidylinositol 3-kinase, and the calcium pathway (55). We have previously shown a critical role for JNK and the upstream SEK1 in IL-12p40 regulation by LPS-activated monocytic cells (13). Because nef-expressing cells exhibited specifically down-regulation of the JNK pathway without any effect on p38 and ERK MAPKs following LPS stimulation, it is reasonable to conclude that CD14 and the LPS-activated downstream signaling molecules, including Myd88 and the interleukin-1 receptor-associated kinase remained unaffected following nef expression.

JNK plays a critical role in Th1/Th2 cell differentiation, cytokines production, and cell survival (56–58). It contains three members, JNK1, -2, and -3. JNK3 is selectively expressed in...
neuronal and cardiac tissues and associated with neuron cell apoptosis, whereas JNK1/2 are highly inducible in monocytes and T cells (56, 57, 59). It is not clear whether JNK1 or JNK2 regulate LPS-induced IL-12p40 expression. Our results suggest that intracellular nef inhibited LPS-activated both JNK1 and JNK2 isoforms in monocytes and THP-1 cells. JNK is activated by SEK1, a dual kinase upstream of JNK MAPKs (44). The results of this study suggest that inhibition of LPS-activated SEK1 may be responsible for the inhibition of JNK activation and subsequent IL-12p40 transcription in both monocytes and THP-1 cells.

JNK is a serine/threonine kinase that activates various transcription factors, including AP1 and NFκB (56). Our results revealed that intracellular nef selectively inhibited NFκB without affecting AP-1 activation. It is not clear if nef inhibited NFκB by inhibiting NFκB-JNK interactions as JNK has been shown to influence NFκB pathway by regulating IkBα activation (60). Because nef has been shown to modulate several signaling molecules, including IkB kinase (33, 34, 52), it is possible that nef may inhibit binding of LPS-activated NFκB to the IL-12p40 promoter by directly affecting the components of the NFκB pathway. However, it remains to be determined if nef inhibits NFκB independent of its interactions with the upstream JNK/SEK1 kinases. JNK can also activate AP-1 mainly via phosphorylation of the c-Jun component (56). However, AP-1 can also be activated independent of the JNK MAPKs (44). The precise mechanism by which intracellular nef inhibits JNK/SEK1 phosphorylation and JNK activation leading to impaired LPS-induced IL-12p40 transcription is not clear. Nef may impair JNK phosphorylation by direct interactions with upstream signaling molecules through the PXXP motif (64–67).

NFκB plays a key role in the development of innate and adaptive immunity and is involved in the pathogenesis of a number of diseases, including cancer, infectious diseases, and inflammatory disorders (68). The biological significance of JNK-mediated activation of NFκB signaling has been documented in cytokine production (IL-6, tumor necrosis factor-α, and MIP-1α) and susceptibility to bacterial and viral infection in animal models (60, 69). In addition, NFκB is a molecular basis for the aberrant growth and cytokine gene expression observed in AIDS (70). Therefore, the inhibition of JNK-mediated NFκB activation by intracellular nef may not only down-regulate IL-12p40 transcription but also be implicated broadly in the development of diseases, Th1 responses, and development of immunodeficiency.

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