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NF-κB Modulates TNF-α Production by Alveolar Macrophages in Asymptomatic HIV-Seropositive Individuals

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Local TNF-α production in different organs may affect HIV replication and pathogenesis. Alveolar macrophages (AMs) obtained by bronchoalveolar lavage from asymptomatic HIV-seropositive and -seronegative individuals did not spontaneously release TNF-α, but LPS stimulation of these cells significantly increased TNF-α production. We tested whether NF-κB affects TNF-α production by AMs using N-tosyl-l-phenylalanine chloromethylketone (TPCK) or N-benzoyl-l-tyrosine ethyl ester (BTEE), which inhibit the degradation of IκB, or tricyclodecan-9-yl-xanthogenate-potassium (D609), which inhibits phospholipase C. Alveolar macrophages were exposed to LPS alone and with the chemical protease inhibitors TPCK, BTEE, and D609. NF-κB DNA binding induced by LPS treatment of AMs was inhibited by TPCK, BTEE, and D609. These agents also inhibited TNF-α mRNA and TNF-α protein production. After 24 h, the levels of TNF-α mRNA reached equilibrium, as assessed by RT-PCR. The levels of NF-κB mRNA remained constant under all conditions. The levels of IκB-α mRNA were similar after 30, 60, and 180 min, but the IκB-β mRNA concentration was initially low and increased over time under all conditions. IκB-α and IκB-β protein production was not affected by the chemical protease inhibitors. Our data show that TNF-α production by LPS-stimulated AMs from asymptomatic HIV-seropositive and -seronegative individuals is regulated via the phospholipase C pathway and by NF-κB DNA binding activity without obvious changes in IκB-α or IκB-β protein concentrations. The Journal of Immunology, 2000, 164: 1588–1594.

The lung is a frequent target organ for complications related to HIV-1 infection (1–3). Alveolar macrophages (AMs), the predominant pulmonary immune cells, mediate first-line host defense, direct pulmonary immune responses, and modulate inflammatory responses in the lungs. Alveolar macrophages are permissive for HIV-1 infection in vitro and may be a major reservoir for HIV-1 (4). Although HIV-1 infection in the lungs is generally latent, HIV-1 replicates in the lungs during advanced AIDS (5), Pneumocystis carinii pneumonia (5), and Mycobacterium tuberculosis infection (6–11).

HIV replication is enhanced in vitro by several of the cytokines that are produced in the lungs of HIV-seropositive patients (12). In particular, TNF-α produced by macrophages may be important in HIV pathogenesis. During M. tuberculosis pneumonia, enhanced HIV-1 replication correlates with increased TNF-α levels in bronchoalveolar lavage (BAL) fluid (10). TNF-α, a proinflammatory cytokine released by AMs, enhances HIV-1 replication by inducing NF-κB DNA binding and by activating the HIV long-terminal repeat in cells other than AMs (13).

NF-κB is an important cellular transcription factor and a crucial component of the host’s response to infection. In most unstimulated cells, NF-κB is retained in the cytoplasm by a specific inhibitor, IκB. A variety of agents can induce phosphorylation-dependent degradation of IκB-α, unmasking the nuclear localization signal on p65 and subsequently activating NF-κB. After activation, IκB dissociates from the complex and triggers the translocation of NF-κB into the nucleus, where it binds to a consensus sequence in the promoter regions of several cellular genes as well as to the HIV long terminal repeat (14–16). Inhibitors of IκB-α degradation often decrease gene activation and reduce the production of inflammatory cytokines (17). N-tosyl-l-phenylalanine chloromethyl ketone (TPCK) modifies the sulfhydryl group in NF-κB, thus preventing its binding to DNA (18). The exact mechanism by which N-benzoyl-l-tyrosine ethyl ester (BTEE) blocks NF-κB binding activity is not fully known. TPCK, but not BTEE, inhibits NF-κB activity and the accompanying IκB-α degradation, most likely by inhibiting the protease responsible for IκB-α degradation (19, 20). Delineation of the mechanism(s) that modulates TNF-α production by AMs may provide important insights into HIV pathogenesis in the lungs. Furthermore, a better understanding of the factors that influence TNF-α release in the lungs may identify potential targets for modifying local HIV replication. The purpose of this study was to characterize the mechanism(s) of TNF-α release by AMs obtained from HIV-seropositive and -seronegative individuals.

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3 Abbreviations used in this paper: AM, alveolar macrophages; TPCK, N-tosyl-l-phenylalanine chloromethyl ketone; BTEE, N-benzoyl-l-tyrosine ethyl ester; D609, tricyclodecan-9-yl-xanthogenate-potassium K; BAL, bronchoalveolar lavage; DIG, digoxigenin; PLC, phospholipase C.
In this paper we describe the use of TPCK, BTEE, and D609 to block TNF-α production by inhibiting NF-κB DNA binding activity in AMs from asymptomatic HIV-seropositive persons. These inhibitors may act at several steps of the pathway between LPS stimulation and TNF-α production. Our data suggest that one such mechanism is the stabilization of the IκB protein, and therefore the IκB-NF-κB complex, with an associated blunting of LPS-induced TNF-α production.

Materials and Methods

Materials

Escherichia coli 0127:B8 LPS was purchased from Difco (Detroit, MI), TPCK and BTEE were purchased from Sigma (St. Louis, MO), and D609 (tricyclodecane-9-yl-xanthogenate-potassium K) was purchased from Bio-omol (Plymouth Meeting, PA).

Study subjects

This study was approved by the institutional review boards of the New England Medical Center and Beth Israel Deaconess Medical Center (Boston, MA). All persons were recruited prospectively, and all participants provided informed consent before participating in the study. Participants had normal spirometric characteristics and did not have active pulmonary disease. For each participant, age, sex, smoking status, medical history, and current medications were recorded on standardized forms. In addition, for the HIV-seropositive individuals, information related to HIV risk factors, peripheral CD4 count, HIV RNA in plasma, medications (including antiretroviral therapy), and HIV-related diseases were recorded.

Each patient completed a comprehensive questionnaire regarding medical history (including details of HIV-1 infection, related infections, and medications) and underwent a physical examination and bronchoalveolar lavage.

Bronchoalveolar lavage

AMs were obtained from patients by bronchoalveolar lavage using standard technique (21). Briefly, after topical 2% lidocaine anesthesia was applied to the oropharynx, a flexible fiberoptic bronchoscope was passed into the airways and wedged in a segment of the right middle lobe. Bronchoalveolar lavage was performed by instilling six to eight 50-ml aliquots of warm nonbacteriostatic saline (0.9%) followed by gentle suction after each aliquot was infused. In general, 75% of the instilled saline was recovered. The lavage fluid was collected into sterile traps in a closed system. The cells were separated from the pooled lavage fluid by centrifugation at 100 × g for 10 min at 4°C, washed in cold RPMI 1640 supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma), and counted on a hemocytometer. Cells were resuspended at 105 cells/ml in RPMI 1640.

Culture of BAL cells

Cells retrieved from HIV-seropositive and -seronegative individuals by bronchoalveolar lavage were obtained by centrifugation at 100 × g for 10 min at 4°C. After centrifugation, the solution was incubated overnight at 4°C with protein A-Sepharose CL-4B (Pharmacia, Piscataway, NJ) for 1 h at 4°C. Supernatant fluids were clarified by centrifugation and stored at −70°C. Then, 2–5 μg of the clarified extracts were used for the shift reaction and incubated for 20 min at room temperature with a NF-κB probe containing the consensus sequence (5′-AGTTGAGGAGCTTCCGAGC-3′; Promega, Madison, WI). Supershift assays were performed with the addition of an Ab directed against p65 (Santa Cruz Biotechnology, Santa Cruz, CA). Abs against p65 was added to the extracts after addition of the probe and incubated for 1 h at room temperature before resolution on a 5% polyacrylamide gel. The probe was end labeled with [γ-32P]ATP. The reaction mixture was analyzed on an 8% polyacrylamide gel run in 0.5× TBE. The gels were dried, and x-ray film was exposed for 16 h.

RNA extraction

Total RNA was extracted in situ using Trizol, as recommended by the manufacturer (Life Technologies). Briefly, 1 ml of Trizol was added to the culture plate, mixed, and incubated for 5 min at room temperature. The solution was transferred to a microcentrifuge tube, and 200 μl of chloroform was added, vortex mixed, and incubated for 10 min. After centrifugation for 5 min, the aqueous layer was transferred to a fresh tube, and RNA was precipitated by adding an equal volume of isopropanol. After centrifugation, the RNA pellet was dried and dissolved in 50 μl of sterile, diethylpyrocarbonate-treated water. The RNA concentration was determined by optical density measurements.

Immunoprecipitation and Western blot

Cytoplasmic extracts were prepared from alveolar macrophages treated with LPS and TPCK, BTEE, or D609. The extracts, with prestained markers (National Diagnostics, Atlanta, GA), were subjected to electrophoresis on a denaturing 10% polyacrylamide-SDS gel, transferred to a nylon membrane (HybOND-N, Amersham, Arlington Heights, IL), and analyzed by Western blot. Blots were blocked with 5% BSA in PBS, followed by incubation with 0.1 μg/ml of primary anti-IκB-α, anti-IκB-β (Santa Cruz Biotechnology), or both for 1 h at room temperature. Anti-rabbit alkaline phosphatase conjugate was the secondary Ab, and the incubation was continued for 45 min at room temperature. Enzymatic activity was detected using vector red (Vector, Burlingame, CA) or 5-bromo-4-chloro-indolyl-phosphate, 4-toluidine salt and nitro blue tetrazolium chloride (Roche, Indianapolis, IN). Alternatively, the extracts were preclarified by incubation with protein A-Sepharose CL-4B (Pharmacia, Piscataway, NJ) for 1 h at 4°C. After centrifugation, the solution was incubated overnight at 4°C with Abs directed against IκB-α.

Immunoprecipitation of the complexes was performed by incubation with 50 μl of protein A-Sepharose, followed by incubation for 4 h at 4°C. After extensive washing, the proteins were solubilized in 40 μl of Laemmli buffer and resolved by SDS-PAGE electrophoresis. Separated proteins were electrophoretically transferred to nitrocellulose, treated with blocking buffer, and incubated for 1 h at room temperature with IκB-α Ab, which was followed, after washing, by incubation with a second alkaline-phosphatase-conjugated Ab. The enzymatic activity was detected using Vector red (Vector) or 5-bromo-4-chloro-indolyl-phosphate, 4-toluidine salt and nitro blue tetrazolium chloride.

RT-PCR

RNA (1–2 μg) was reverse transcribed using the kit Advantage RT for PCR (Clontech, Palo Alto, CA) according to the manufacturer’s instructions. The final cDNA product was diluted to 100 μl with sterile diethylpyrocarbonate-treated water. An aliquot of the cDNA was used for specific cytokine PCR using the appropriate TNF-α, IκB-α, IκB-β, and actin primers (CLP, Clontech or Tufts University Core Synthesis Facility). The conditions for amplification reactions were as described by the manufacturer for the aliquot of the cDNA product. For amplification, each 50 μl reaction mixture was denatured at 158°C for 15 s and then heated at 95°C for 15 s, followed by 20 cycles of 15 s at 95°C, 15 s at 58°C, and 1 min at 72°C, with a final extension of 5 min at 72°C. For the RT-PCR experiments, the DNA was stained with 5 μl of ethidium bromide (1%) and photographed. PCR primers for actin were used as an internal control.
In some cases, the sense primer was 5’ end labeled with 32P. After electrophoresis, the gel was dried, and autoradiography was performed.

**PCR and flow cytometry**

Nonadherent cells and AMs were separated by adhesion to plastic for 16 h at 37°C and resuspended in 50 μl of 4% paraformaldehyde in PBS for 30 min at 4°C. The cells were washed in PBS, incubated for 5 min at 37°C in a solution of 120 μg/ml of pronase, briefly incubated at 95°C for 3 min, and centrifuged. The cell pellet was resuspended in a mixture containing 200 pmol of primers (SK38 and SK39)/reaction; 200 μM each of dGTP, dATP, and dCTP; 196 μM dTTP; 4 μM dUTP-11-DIG (Roche), 40 U of Rnasin (Promega), and 2.5 U of Taq polymerase. The cycling conditions were as follows: denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 1 min, for a total of 35 cycles. The samples were centrifuged and washed extensively before being incubated for 30 min at room temperature with anti-DIG- FITC (1/1000; Roche), washed, and analyzed by flow cytometry using a Becton Dickinson flow cytometer (Mountain View, CA).

**Results**

Cytokine and chemokine production by AMs from HIV-seropositive and -seronegative subjects differed between lung and peripheral blood cells (J.-M. Mathys, manuscript in preparation). Spontaneous TNF-α production by AMs from HIV-seropositive patients was negligible, but increased after LPS stimulation. TNF-α protein production was measured by ELISA in the supernatant fluids after 24 h. Spontaneous TNF-α production by AMs from HIV-seropositive (n = 11) and -seronegative (n = 6) subjects was very low (mean, 196 and 176 pg/ml, respectively). However, after LPS stimulation, BAL cells from HIV-seronegative subjects produced more TNF-α than those from asymptomatic HIV-infected subjects (mean, 7018 compared with 3766 pg/ml; p = 0.012, by nonparametric Wilcoxon matched pair, signed ranks test for paired samples). In this study we analyzed the production of TNF-α by AM and the roles of NF-κB and IκB in this process.

**Effects of TPCK and BTEE on TNF-α production by AM**

We studied the effects of TPCK and BTEE (serine proteases) on the production of TNF-α by AMs. To determine whether the chemical protease inhibitors act in a dose-dependent manner, we analyzed the responses of AMs after incubation with various concentrations of chemical protease inhibitors. Cells from patients (n = 3) were cultured for 16 h in plastic tissue culture plates. The nonadherent cells from BAL were removed, and TPCK and BTEE were added to AMs at various concentrations in 2 ml of fresh medium.

Treatment of AMs with TPCK and BTEE caused a dose-dependent inhibition of TNF-α production with almost complete inhibition at 50 μg/ml (Fig. 1, A and B). Cell viability, as determined by vital dye exclusion (trypan blue), was always >85% after incubation of AMs for 24 h with the chemical protease inhibitor at the concentrations used. We next determined the effects of TPCK, BTEE, and D609 on TNF-α production from AMs from HIV-seropositive subjects and HIV-seronegative controls. LPS induced TNF-α production after 24-h culture, but TPCK and BTEE almost completely inhibited this effect in both HIV-seropositive and -seronegative subjects (Fig. 2).

**Effects of TPCK and BTEE on TNF-α and NF-κB mRNA levels**

To correlate protein production with TNF-α mRNA and NF-κB mRNA concentrations, RNA was extracted after 4 and 24 h. The levels of TNF-α and NF-κB mRNAs were measured after 24-h incubation by RT-PCR and were detected by ELISA. The results were normalized for actin mRNA. Adding TPCK and BTEE to AMs rapidly (4 h) inhibited TNF-α mRNA production, in contrast to the level of production obtained after LPS stimulation alone (Fig. 3). TNF-α mRNA production was almost completely inhibited by these drugs at 4 h. After 24-h incubation, the levels of mRNA differed little between LPS-stimulated AMs treated with the chemical protease inhibitors and controls. The levels of NF-κB mRNA in treated AMs were comparable to the levels obtained in LPS-stimulated AMs (data not shown). These results suggest that the chemical protease inhibitors decreased TNF-α mRNA at 4 h, but did not affect NF-κB mRNA.

**Effects of phospholipase C (PLC) signaling pathway inhibitors on TNF-α production by alveolar macrophages**

Because NF-κB activation is closely linked to the PLC signaling pathways, we studied the effects of an inhibitor (D609) of PLC on LPS-induced TNF-α production by AMs from asymptomatic HIV-seropositive individuals. Adding D609 (50 μg/ml) to AMs almost completely inhibited LPS-stimulated TNF-α production by AM (Fig. 2), suggesting a link between NF-κB activation and the PLC pathway and indirectly with TNF-α production.

**Effects of TPCK and BTEE on NF-κB activity in AMs**

Various combinations of NF-κB proteins form active NF-κB heterodimers that bind to specific sequences in DNA. To determine whether the retarded bands by EMSA in untreated and treated cells were indeed NF-κB, cell extracts were incubated with Ab against p65. To analyze the effects of adding TPCK and BTEE on the DNA binding of NF-κB in AMs, nuclear extracts were prepared after various treatments of the AMs and were analyzed by EMSA. NF-κB binding in untreated, unstimulated AMs (Fig. 4) was spontaneously detectable (lane 2), and it was enhanced by LPS stimulation (lane 3). After treating LPS-stimulated AMs with TPCK
and BTEE, DNA binding in the nuclear extracts decreased substantially (lanes 4 and 5). The specificity of the binding was confirmed by competition with an excess of cold probe and by competition with an irrelevant probe (SP1; Fig. 4). Adding Ab against p65 resulted in a supershift, suggesting that the complex contains p65 subunits (Fig. 5).

**Effects of the chemical proteases on IκB-α and IκB-β**

To examine the potential effects of TPCK, BTEE, and D609 on IκB at the RNA and protein levels, RNA was extracted, and cytosolic extracts were prepared after 30, 60, and 180 min of treatment of the cells with LPS, TPCK, and BTEE. RNA was analyzed by RT-PCR with primers specific for IκB-α and IκB-β. IκB-α mRNA concentrations did not vary between 30 and 180 min, and there was no detectable difference in the concentration between untreated and unstimulated AMs and LPS-stimulated and treated macrophages. In contrast, IκB-β mRNA was barely detectable at 30 min, but it increased over time and was detected at 180 min under all culture conditions (Fig. 6).

The extracts were analyzed by immunoprecipitation and immunoblotting using polyclonal Abs against IκB-α (Santa Cruz Biotechnology). IκB-α protein was detected at 30 min in unstimulated, LPS-stimulated, and LPS-stimulated cells treated with TPCK and BTEE (Fig. 7A). The nonphosphorylated protein was detected, but its concentrations did not differ under the various conditions tested. IκB-β protein was detected in unstimulated AMs and LPS-stimulated cells with or without protease inhibitors. The concentrations of IκB-α and IκB-β proteins did not differ over time (Fig. 7B).

**In situ PCR of AMs and nonadherent cells**

To confirm HIV infection of lung cells used in these experiments, AMs and nonadherent cells were separated by plastic adherence and processed by PCR in suspension with HIV-specific primers.
NF-κB MODULATES TNF-α PRODUCTION BY ALVEOLAR MACROPHAGES

FIGURE 5. The results of supershift EMSA. Supershift EMSA was performed with the addition of anti-p65 and was followed by incubation for 1 h at room temperature and then by electrophoresis on a native 5% gel. The dried gels were exposed to x-ray film for 16 h. Lane 1, unstimulated cells; lane 2, unstimulated cells and anti-p65; lane 3, LPS-stimulated cells; lane 4, LPS-stimulated cells and anti-p65. The arrow shows the positions of the supershifted bound complexes.

FIGURE 6. The effects of the chemical protease inhibitors on IκB-α and IκB-β mRNA in AMs from HIV-seropositive subjects. AMs (5 × 10^7/well) were incubated with LPS in the presence of the chemical protease inhibitors. RNA was extracted from the various specimens after 30, 60, and 180 min and analyzed for IκB-α and IκB-β mRNA by RT-PCR. The amplified products were resolved on an 8% native polyacrylamide gel and stained with 1% ethidium bromide. The large arrow indicates IκB-α, and the small arrow indicates IκB-β. Lane 1, molecular mass markers; lane 2, AMs and medium alone (30 min); lane 3, AMs and LPS; lane 4, AMs, LPS, and TPCK (30 min); lane 5, AMs, LPS, and BTEE (30 min); lane 6, AMs, LPS, and D609 (30 min); lane 7, AMs and medium alone (60 min); lane 8, AMs and LPS (60 min); lane 9, AMs, LPS, and TPCK (60 min); lane 10, AMs, LPS, and BTEE (60 min); lane 11, AMs, LPS, and D609 (60 min); lane 12, AMs and medium alone (180 min); lane 13, AMs and LPS (IκB-α omitted; 180 min); lane 14, AMs, LPS, and TPCK; lane 15, AMs, LPS, and BTEE.

Discussion

We describe the use of TPCK, BTEE, and D609 to block TNF-α production by inhibiting NF-κB DNA binding in human alveolar macrophages from asymptomatic HIV-seropositive and -seronegative individuals. TPCK, BTEE, and D609 may act on one or more of the many intermediary steps in a complex signaling pathway leading to TNF-α production.

Stimulating AMs with LPS leads to the rapid expression of many genes that encode cytokines. The importance of NF-κB in controlling this expression has been demonstrated in several systems (23), including patients with acute respiratory distress syndrome (24). NF-κB binds to DNA as a dimer. The most prevalent NF-κB dimer consists of the two subunits, p65 and p50, and is present in the cytosol in an inactive form bound to inhibitory proteins collectively named IκB (IκB-α, -β, and -ε). After activation of the cells, a cascade of events leads to NF-κB dissociation from IκB (25). The activation of NF-κB is correlated with the phosphorylation of IκB and its subsequent degradation. The loss of IκB in the cytoplasm is followed by the appearance of NF-κB in the nucleus. Although phosphorylation of IκB occurs before its degradation, recent studies show that phosphorylation of IκB alone does not release active NF-κB (17).

Inflammatory cytokines, such as TNF-α and IL-1, are produced by AMs and may affect HIV pathogenesis. Although NF-κB regulatory sequences have been found in the promoter regions of TNF-α and IL-β, there is still debate concerning the extent to which NF-κB is required for the expression of these genes (26).

In these experiments we used two chemical chymotrypsin-like protease inhibitors (TPCK and BTEE) that prevent NF-κB translocation to the nucleus and therefore reduce TNF-α production. Incubation of AMs for 24 h in the presence of TPCK and BTEE significantly decreased the production of TNF-α by AMs, and this decrease was dose dependent. These compounds reduced TNF-α production by AMs to constitutive levels. A similar effect has been described using murine AMs (27).

The factors controlling TNF-α release by AMs during HIV-1 infection are not completely defined. LPS-induced cytokine relea-
IκB-α and IκB-β concentrations did not differ substantially among the unstimulated, LPS-stimulated, and chemically treated specimens, as assessed by immunoblotting using polyclonal anti-IκB-α and anti-IκB-β Abs. This lack of difference suggests that TPCK and BTEE do not affect the level of expression of IκB-α and IκB protein. The specific functions of IκB-β are not yet clear. In endothelial cells, a transient phase of NF-κB activation may be mediated through IκB-α, and a more sustained phase may be mediated through IκB-β, because IκB-α is degraded more rapidly than IκB-β (29–31).

Protease inhibitors (such as TPCK and BTEE) block LPS-induced degradation of IκB-α and IκB-β and inhibit NF-κB binding. At the mRNA level, they block the expression of TNF-α and IL-1β in monocytic cells (32). The chemical proteases inhibitors rapidly reduced TNF-α mRNA expression in AMs. TNF-α mRNA was inhibited as soon as 4 h after treatment; at 24 h, there was little difference in the concentration of TNF-α mRNA compared to the pretreatment level. The mechanism responsible for the reappearance of TNF-α mRNA after 24 h is not known. Conceivably, the chemical protease inhibitors are no longer active after 24 h. In B cells (70Z/3 cells), TPCK inhibited NF-κB binding activity as well as IκB-α decay in response to PMA treatment (19). In AMs, addition of TPCK, BTEE, or both did not affect the expression of IκB-α after 2, 4, or 24 h.

Our results show that inhibiting PLC with a specific inhibitor, D609 (33), significantly reduced TNF-α expression among LPS-stimulated macrophages in a dose-dependent manner. D609 is a complete inhibitor of acidic sphingomyelinase as well as PLC (34). A major pathway activated by tyrosine kinases involves PLC.

NF-κB activation by LPS is closely linked to the PLC signaling pathways. The PLC pathway is critical to the production of TNF-α (35). One study of AMs from HIV-seronegative subjects showed that these events were protein kinase C-independent and PLC-tyrosine kinase-dependent (36). Another group found that protein kinase C inhibitors (staurosporine and sphingosine) suppressed LPS-induced TNF-α production in AMs and in whole blood (40–90%) from HIV-seropositive subjects. AMs were less sensitive to protein kinase C inhibition than blood monocytes (37).

In conclusion, spontaneous TNF-α release by AM from asymptomatic HIV-1-infected persons is no greater than that from healthy persons. After LPS stimulation, TNF-α release in the supernatant fluids increased, and the process involved the induction of NF-κB DNA binding associated with the PLC signaling pathway. These observations provide important insights into the mechanisms of TNF-α release in the lungs of HIV-infected subjects. These findings may have implications concerning the pathogenesis of HIV-1 infection and the regulation of HIV-1 replication in the lungs of subjects with HIV-1 infection. Therapeutic strategies aimed at modifying the release of TNF-α by AM may limit the local replication of HIV-1 in the lungs and prevent the pulmonary complications associated with HIV-1 infection.

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