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Human immunodeficiency virus-1-tat induces matrix metalloproteinase-9 in monocytes through protein tyrosine phosphatase-mediated activation of nuclear transcription factor NF-κB

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Abstract We have previously shown that human immunodeficiency virus (HIV)-1-tat induces the production of matrix metalloproteinase-9 (MMP-9) in human monocytes by a mechanism that is not understood. In the present report, we demonstrate that HIV-tat-induced expression of MMP-9 is blocked by inhibitors of protein tyrosine phosphatases (PTPases). PTPase inhibitors also blocked HIV-tat-induced nuclear transcription factor NF-κB activation and IκBα degradation required for MMP-9 induction. These results suggest that HIV-tat induces MMP-9 in human monocytes through activation of PTPase and NF-κB.

Key words: Human immunodeficiency virus-tat; Monocyte; Nuclear factor-κB; Metalloproteinase; Protein tyrosine phosphatase

1. Introduction

Monocytes are major targets of human immunodeficiency virus-1 (HIV-1) infection and serve as a reservoir of viral replication [1,2]. HIV infection enhances monocyte production of IFN-γ, IL-1β, IL-6, GM-CSF, TNF and extracellular matrix (ECM)-degrading metalloproteinases [3–7]. In addition HIV-infected monocytes have a defect in the presentation of antigen to CD4+ T cells [8]. Several effects of HIV infection of monocytes are mimicked by HIV-tat, a 76 aa protein of HIV origin [9–11]. Exogenous HIV-tat acts as a potent transactivator of HIV-1 long terminal repeat (LTR) and is essential for the replication of the virus [12]. Recently we have shown that treatment of monocytes with soluble HIV-tat protein enhances monocyte production of matrix metalloproteinases (MMP-9) [9,10]. The MMPs compose a family of structurally related endopeptidases that resorb macromolecules of the extracellular matrix. They are produced mainly by cells of the myeloid lineage. Involved in normal tissue remodeling and wound repair, in excess they may lead to connective tissue damage and dysfunction [13,14]. Elevated MMP-9 production contributes to the extravasation of HIV-infected monocytes through vascular endothelium into the tissue in the early stages of HIV disease [7,10,12]. However, the mechanism by which HIV-tat induces the production of MMP-9 is not known.

One possibility is through the nuclear transcription factor-κB (NF-κB), a pleiotropic regulator of many genes involved in immune and inflammatory responses [15]. The activation of NF-κB requires dissociation of a cytoplasmic heterodimer consisting of p50 and p65 polypeptides from the inhibitory subunit known as IκB. The dissociation of IκB is followed by its degradation and the translocation of the p50-p65 heterodimer to the nucleus [16]. HIV-tat has been shown to activate NF-κB in some cell types [17], but whether it activates NF-κB in monocytes and whether such activation leads to MMP-9 production is not known. Further, although HIV-tat has been reported to modulate the activity of several kinases involved in signal transduction pathways, the role of protein phosphatases (PPase) in HIV-tat-mediated cell signaling has remained unclear. Using metabolic inhibitors of protein tyrosine phosphatases (PTPase), we have previously shown that tumor necrosis factor (TNF)-induced cell surface expression of adhesion molecules and NF-κB activation in microvessel endothelial cells is regulated by PTPase [18,19]. In the present study we report that PTPase are also involved in HIV-tat-mediated activation of NF-κB and MMP-9 production.

2. Materials and methods

2.1. Materials

Penicillin, streptomycin, RPMI 1640 medium, and fetal bovine serum were obtained from Gibco (Grand Island, NY). Glycine, NaCl, and bovine serum albumin (BSA), diamide, and sodium orthovanadate were obtained from Sigma Chemical Co. (Saint Louis, MO); phenylarsinine oxide (PAO) was obtained from Aldrich Chemicals (Milwaukee, WI). HIV-tat protein was obtained from the AIDS Research and Reference Reagent Program of the National Institute of Allergy and Infectious Diseases (Rockville, MD). U937 (a monocytic cell line) cells were obtained from the American Type Culture Collection (Rockville, MD).

2.2. Detection of metalloproteinase in monocyte-conditioned medium

U937 were suspended at 10^6 cells/ml in RPMI containing 5% FBS with or without HIV-tat protein for 24 h. The cells were then washed in RPMI medium and resuspended at 1×10^6 cells/ml and incubated for an additional 16 h. The cells were pelleted by centrifugation, and the conditioned media collected for analysis by gelatin zymography [10]. Aliquots of the conditioned media collected were mixed with

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Abbreviations: MMP-9, metalloproteinase-9; NF-κB, nuclear factor-κB; PTPase, protein tyrosine phosphatases; HIV-tat protein, human immunodeficiency virus-tat protein; PAO, phenylarsine oxide; PV, pervanadate; DTT, dithiothreitol; EMSA, electrophoretic mobility shift assay

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sample buffer and subjected to electrophoresis using 10% polyacrylamide gel containing SDS and 1 mg/ml gelatin without prior heating or reduction. The gels were incubated in 2.5% Triton X-100 for 1 h to remove SDS and then incubated for 24 h in 50 mM Tris-HCl, pH 7.6, 50 mM NaCl, 5 mM $CaCl_2$, and 0.02% NaN_3. Finally, the gels were stained for 60 min in 30% methanol, 10% acetic acid, and 0.5% Coomassie blue R-250, and then destained in distilled water.

2.3. Electrophoretic mobility shift assay (EMSA) for NF-κB

EMSAs were carried out as described in detail previously [18].

2.4. Western blotting for IκB

After the NF-κB activation reaction described above, postnuclear extracts were resolved on 9% SDS-polyacrylamide gels, electrotransferred to nitrocellulose paper, probed with rabbit polyclonal antibody against IκBα, and detected by chemiluminescence (ECL, Amersham Corp.) [18].

3. Results and discussion

In this study we used inhibitors of protein tyrosine phosphatases to examine the role of protein tyrosine phosphorylation in the HIV-tat-mediated induction of MMP-9 production. For this study U937 cells were used because these monocytic cells produce MMP-9 upon appropriate stimulation [20,21]. The time of incubation and the concentrations of HIV-tat and inhibitors used in this study had no effect on the viability of the cells (data not shown).

3.1. Effect of protein tyrosine phosphatase inhibitors on the production of MMP-9 by U937 cells

In a recent report we showed that treatment of human monocytes with HIV-tat protein resulted in a dose- and time-dependent increase in the production of MMP-9 in the cultures [10]. We used PAO, pervanadate (PV), and diamide as PTPase inhibitors ([18] and references therein) and investigated their effect on the production of MMP-9 in response to HIV-tat. U937 cells were first pretreated with PAO, PV, or diamide for 30 min and then with 100 ng/ml HIV-tat protein at 37°C for 24 h, followed by incubation of the cells for another 18 h in medium. MMP-9 production was assayed as described in Section 2. Treatment of U937 cells with HIV-tat enhanced production of MMP-9 in the conditioned medium; the increase in MMP-9 was completely blocked by PAO, PV, and diamide. The inhibitors had no effect on the production of MMP-9 by cells that were not treated with HIV-tat (Fig. 1).

3.2. HIV-tat activates NF-κB in U937 cells

Because the promoter region of MMP-9 contains NF-κB binding sites [22], we next investigated the effect of HIV-tat protein on the activation of NF-κB in U937 cells. The cells were treated with 100 ng/ml HIV-tat for variable times and NF-κB activation was measured. Maximum activation of NF-κB was seen after 1 h of treatment with HIV-tat (Fig. 2A). Treatment of cells with different concentrations of HIV-tat protein for 1 h at 37°C activated NF-κB in a dose-dependent manner, reaching a maximum at a concentration of 100 ng/ml (Fig. 2B).

To show that the retarded band observed by EMSA in HIV-tat-treated cells was indeed NF-κB, nuclear extracts were incubated with antibodies either to p50 (NF-κB1) or to p65 (RelA) subunits. The antibodies to either subunit of NF-κB shifted the band to a higher molecular weight (Fig. 2C), thus suggesting the HIV-tat-activated complex consisted of

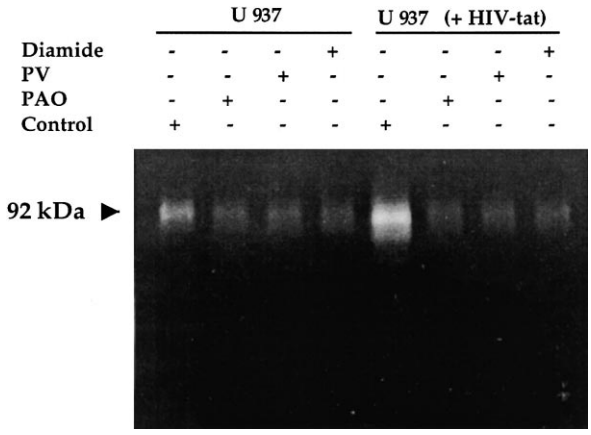

Fig. 1. Effects of PAO, PV and diamide on HIV-tat-induced production of MMP-9 by U937 cells. U937 (1×10^6) cells were pretreated with or without PAO (2.4 μM), PV (50 μM), or diamide (0.5 mM) for 30 min and then with 100 ng/ml HIV-tat protein or treatment buffer alone for 18 h at 37°C in a CO_2 incubator. The cells were washed and then incubated in serum-free RPMI medium for an additional 16 h. The cells were then collected by centrifugation, and an aliquot of cell-free supernatant was subjected to electrophoresis using 10% polyacrylamide gel containing SDS and 1 mg/ml of gelatin. The gels were treated with 2.5% Triton X-100, developed for 24 h, and stained with Coomassie blue.

p50 and p65 units. Since only a partial shift of the NF-κB band was observed with anti-p50 antibody, it is possible that the NF-κB complex also consists of p52-p65 heterodimers. Non-specific antibody against cyclin D or preimmune serum had no effect on the mobility of NF-κB.

3.3. HIV-tat-induced activation of NF-κB involves the degradation of IκBα

Activation of NF-κB by many agents requires dissociation of inhibitory protein IκBα, which then undergoes proteolytic degradation [15,16]. To determine the effect of HIV-tat on the cellular level of IκB, U937 cells were treated with 100 ng/ml of HIV-tat protein for different times and the cytoplasmic extracts were examined by Western blot analysis using IκB-specific antibodies. HIV-tat sharply decreased the level of IκB after 45 min of treatment; the protein reappeared after 90 min, presumably because of NF-κB-dependent resynthesis (Fig. 2D). These results thus show that the activation of NF-κB by HIV-tat coincides with its effect on the degradation of IκB, the inhibitory protein.

3.4. Protein tyrosine phosphatase inhibitors block the HIV-tat-induced activation of NF-κB

PAO has been shown to be a specific inhibitor of PTPase. As PAO was shown to block the production of MMP-9 by U937 cells, we determined the effect of PAO on HIV-tat-mediated activation of NF-κB. The U937 cells were incubated with different concentrations of PAO for 30 min and then treated with HIV-tat (100 ng/ml) for 1 h; and activation of NF-κB was checked. As shown in Fig. 3A, cotreatment of U937 cells with PAO inhibited the activation of NF-κB in a dose-dependent manner. The effect of another inhibitor of PTPase, PV, on HIV-tat-induced activation of NF-κB is shown in Fig. 3B. A 50 μM concentration of PV was sufficient to completely inhibit the activation of NF-κB in response to HIV-tat. However, a slight activation of NF-κB was seen at 10 μM in this and two other independent experiments (data not shown).

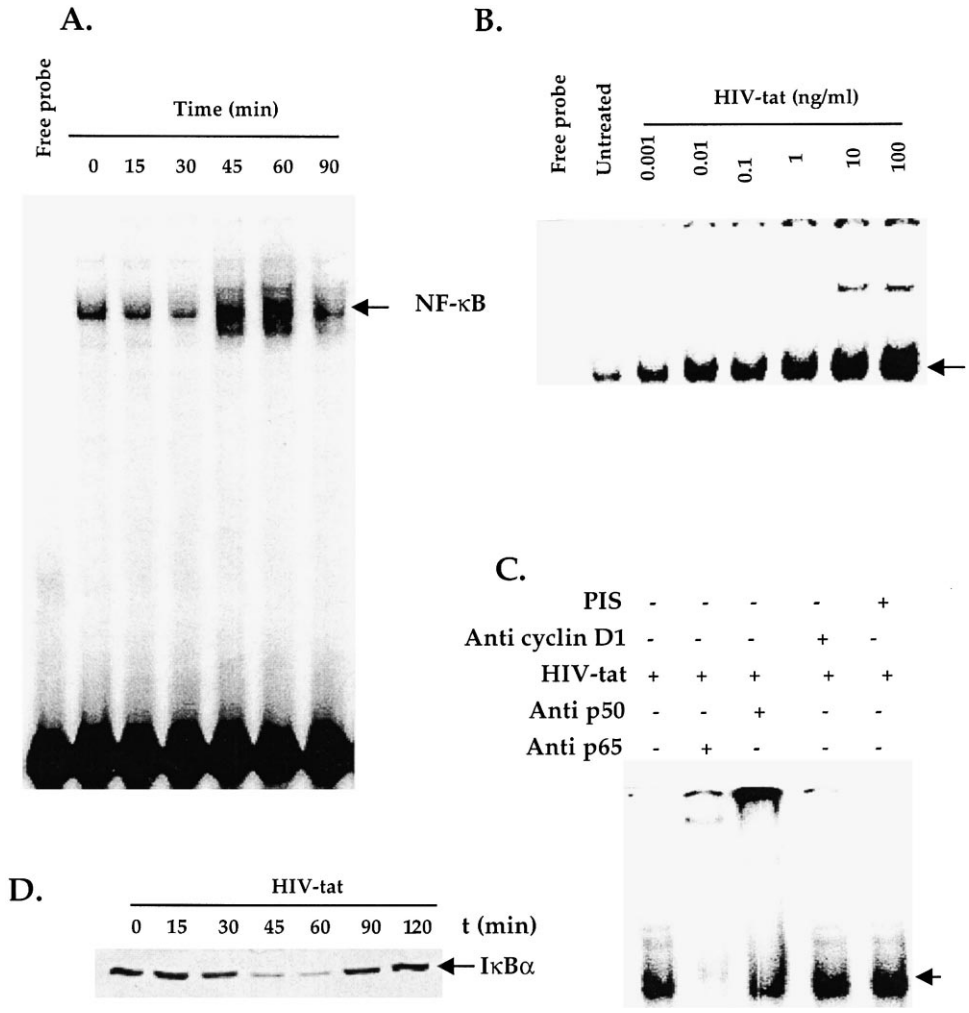

Fig. 2. Effect of HIV-tat protein on activation of NF-κB. A: The cells were treated with 100 ng/ml HIV-tat protein for different intervals of time at 37°C. B: U937 (10^6) cells were treated with indicated concentrations of HIV-tat protein for 1 h. C: Nuclear extracts were prepared from 100 ng/ml HIV-tat-treated U937 cells and incubated with different antibodies for 30 min at room temperature and then nuclear extracts were assayed for NF-κB as described in Section 2. D: Effect of HIV-tat protein on degradation of IκB. U937 (10^6) cells were treated with 100 ng/ml HIV-tat protein for different intervals of time, and the cytosolic extracts were assayed for IκB by Western blot analysis as described in Section 2.

Whether PTPase inhibitors block NF-κB activation by blocking HIV-tat-induced IκBα degradation was also examined. To determine this U937 (1×10^6) cells were pretreated with PAO for 30 min and then treated with 100 ng/ml HIV-tat protein for different intervals of time, and the cytosolic extracts were assayed for IκB by Western blot analysis. The results clearly show that HIV-tat-induced degradation of IκBα is inhibited by pretreatment of cells with PAO (Fig. 3C).

This is the first report to document the role of PTPase in the production of MMP-9 by human monocytes in response to HIV-tat protein. In another, earlier, report, we have shown that PTPase plays an important role in the activation of NF-κB by TNF [18]. Since the promoter region of the MMP-9 gene contains NF-κB binding sites [22], which play a critical role in the expression of this gene, we investigated the effects of HIV-tat protein on the activation of NF-κB and its modulation by the inhibitors of PTPase in human monocytes. We have recently shown that the expression of cell surface adhesion molecules in human endothelial cells is regulated by a similar mechanism [19] involving the activation of NF-κB. It is therefore possible that HIV-tat treatment of cells induces a signal transduction cascade that ultimately results in the activation of NF-κB and the expression of the plethora of genes involved in inflammatory responses. Indeed, HIV-tat has been shown to upregulate the expression of several growth regulatory molecules such as lymphotoxin, TNF, interleukin-2, transforming growth factor-β and major histocompatibilty complex class I molecules, all of which have DNA binding sequence for NF-κB near their promoter region [9–11,15].

Our results suggest that PTPase inhibitors suppress NF-κB activation by blocking IκBα degradation. These results are similar to that reported by us and others for abrogation of TNF-induced NF-κB activation by PTPase inhibitors [18,23,24]. PV is known to induce phosphorylation of tyrosine residue 42 in IκBα which prevents its phosphorylation at serine 32 and 36 and subsequent degradation [23]. HIV-tat is known to activate NF-κB in not only monocytes but also non-monocyte cells [25–28]. The role of NF-κB in HIV-1 gene expression and replication has been well established. NF-κB pathways play a central role in HIV-1 LTR-driven transcription. The HIV-1 LTR contains two adjacent κB binding sites in the enhancer region [29–31]. Transient trans-

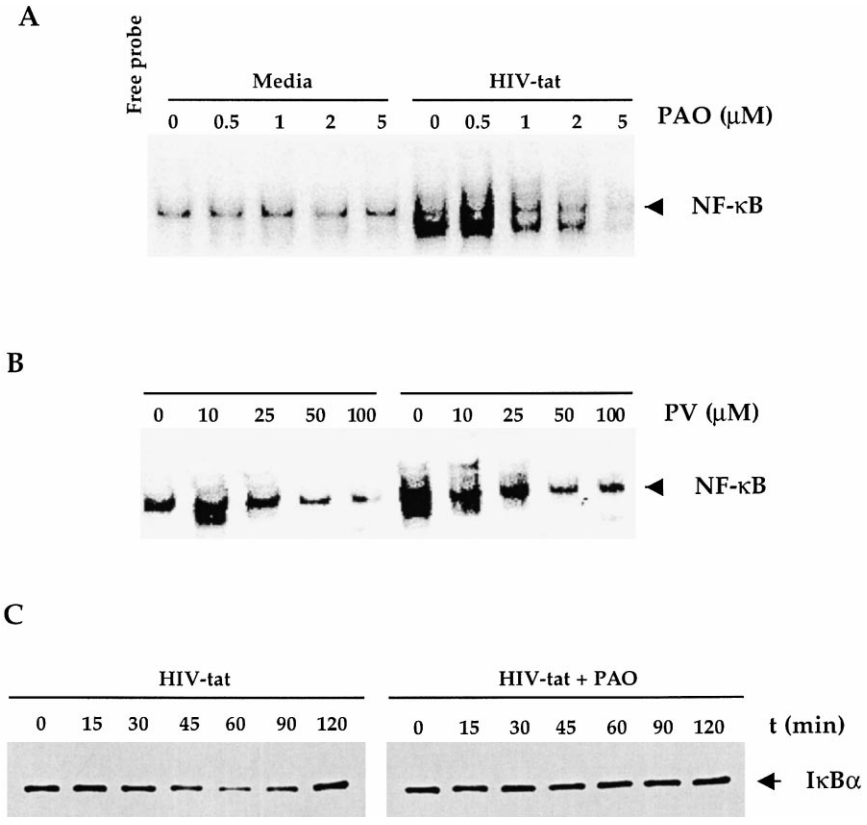

Fig. 3. Effect of PTPase inhibitors on the activation of NF-κB in response to HIV-tat protein. U937 (10^6 cells/ml) were incubated with indicated concentrations of (A) PAO and (B) PV for 30 min and then treated with HIV-tat (100 ng/ml) for 1 h at 37°C. The nuclear extracts were then made and assayed for NF-κB. C: Effect of PAO on HIV-tat-induced degradation of IκB. U937 (10^6) cells were pre-treated with PAO for 30 min and then treated with 100 ng/ml HIV-tat protein for different intervals of time, and the cytosolic extracts were assayed for IκB by Western blot analysis as described in Section 2.

fection studies with HIV-1 LTR or HIV-1 enhancer reporter constructs showed that HIV-1 gene expression increases upon induction of NF-κB binding activity, and a mutation in the NF-κB motif reduced gene expression in the presence or absence of different stimulators [32,33]. Since HIV-tat also increases the activation of NF-κB and MMP-9, it may synergistically contribute to the pathogenesis of HIV. Pathogenesis could in turn be blocked by the inhibitors of PTPases. Whether blockers of NF-κB activation modulate the HIV-mediated pathogenesis remains to be established.

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