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Pertussis Toxin B-Oligomer Suppresses IL-6 Induced HIV-1 and Chemokine Expression in Chronically Infected U1 Cells via Inhibition of Activator Protein 1

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Pertussis toxin (PTX),3 the main pathogenic protein released from Bordetella pertussis, is composed of an A promoter, responsible for cellular toxicity, and a nontoxic B-oligomer (PTX-B) that binds to an as yet unidentified receptor(s) on the surface of target eukaryotic cells (1). PTX-B mediates the toxic activity of the holotoxin, facilitating translocation of the A promoter across the cell membrane (2). Independently of this function, PTX-B triggers cellular signaling in terms of increased levels of diacylglycerol, protein kinase C, and MAPK activation as well as Ca2+ fluxes (3–6). Thus, several biological effects previously ascribed to the holotoxin are indeed exerted by PTX-B (1, 7).

Concerning HIV infection, both PTX and PTX-B have been shown to prevent entry of CCR5-dependent (R5), but not CXCR4-dependent (X4), HIV-1 in activated PBMC and monocyte-derived macrophages (MDM) (3, 8, 9). In addition, PTX-B inhibited the replication of both R5 and X4 HIV-1 strains by acting at one or more postentry mechanisms in activated primary T cells, MDM (3, 10), and chronically infected cell lines, such as the promonocytic U1 cell line stimulated with PMA or cytokines (9, 11). In this regard, different cytokines can up-regulate HIV replication in these different models of acute and chronic infection (3, 10). Among others, IL-6 has been shown to induce HIV replication in MDM infected in vitro (12, 13) and in resting memory CD4+ T cells of infected individuals in combination with TNF-α and IL-2 (14). Unlike TNF-α, IL-6 was previously shown to up-regulate virus production in U1 cells independently of NF-κB activation (12). Functionally, IL-6 stimulation of both MDM and the U937 promonocytic cell line (from which the U1 cell line was derived) induced synthesis and secretion of chemokines such as CCL2/ MCP-1 and CXCL8/IL-8 (15, 16), which are capable of promoting HIV replication in activated PBMC and MDM (15–17).

In the present study we have investigated the molecular pathways through which PTX-B regulates HIV expression in chronically infected U1 and IL-6-responsive U1-derived CR1 cells stimulated with this cytokine.

Materials and Methods
Reagents
Biochemically purified PTX-B (Calbiochem) was dissolved at 50 ng/ml in sterile PBS (BioWhittaker) and used at a final concentration of 75 ng/ml (corresponding to 1 nM), unless otherwise indicated; PMA and GM-CSF were used at final concentrations of 6.16 pg/ml (10−8 M) and 140 pg/ml, respectively (18); LPS (Escherichia coli 0127:B8) final concentration, 2 ng/ml was purchased from Sigma-Aldrich; TNF-α, IL-6, IFN-γ, and IL-1β were purchased from R&D Systems and used at final concentrations of 1, 10, 50, and 100 ng/ml, respectively, according to previous studies (9, 18–21).

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U1 and U1-CRI chronically infected cell lines

The U1 cell line was originally derived from U937 promonocytic cells acutely infected with the X4 laboratory-adapted strain HIV-1LAI (subtype B) and contains two copies of integrated provirus per cell (22, 23). Its state of relative viral latency is mostly dependent upon a defective Tat/Tat region interaction (24, 25). Indeed, transfection of a Tat-expressing plasmid (10, 22, 24) or incubation of U1 cells with exogenous Tat protein (25, 26) rescues viral production. Minimal to undetectable levels of HIV are produced by unstimulated U1 cells, although robust virion release can be promptly induced in these cells upon stimulation with phorbol esters, such as PMA (27), and several proinflammatory cytokines (28, 29). For this reason, this cell line has served as a useful model to identify cytokines and other factors capable of modulating HIV expression and replication in primary cells in vitro and ex vivo (28, 30, 31). At the molecular level, in addition to Tat (32), several cellular transcription factors with binding sites in the HIV-1 long-terminal repeats (LTR) or present in the HIV genome (33) can be activated by chemical agents such as ionomycin (34) and PMA (35), superinfection by other viruses (36, 37), or cytokine stimulation (28, 29). Among these latter, IL-6 was early identified as a potent HIV-inducive cytokine acting by a mechanism different from that triggered by TNF-α or PMA (i.e., activation of NF-κB and consequent HIV-1 LTR-driven transcription) (12). In the present study, U1 and U1-derived CR1 cells (see below) were typically seeded at 2 × 10⁶ cells/ml in RPMI 1640 containing 10% FCS, 1% glutamine, and 1% penicillin/streptomycin in the presence or the absence of the indicated stimuli.

Cytofluorometric analysis

IL-6R expression was measured on 10⁶ U1 and U1-derived CR1 cells stained with IL-6 by the Fluorokine method (R&D Systems) following the manufacturer’s instructions. Cells were then analyzed with a FACSscan apparatus (BD Biosciences) using CellQuest software (BD Biosciences).

Western blot analysis

Ten micrograms of cellular proteins, quantified by Bradford assay (Bio-Rad), was separated by 10% SDS-PAGE, transferred onto nitrocellulose membrane (Hybond ECL; Amersham Biosciences), and incubated with 5% BSA/TBS and 0.2% Tween 20 for 18 h at 4°C. Binding of Abs was visualized by HRP-conjugated Ab (Promega), and the signal was revealed by ECL according to the manufacturer’s instruction (Amersham Biosciences). Images were acquired with a Kodak EDAS 290 (Eastman Kodak) system, and optical blot density was analyzed by Kodak 1D software. Ab anti-p-STAT3 and STAT3 were both obtained from Santa Cruz Biotechnology.

Nuclear cell extracts (NCE) and EMSA

NCE were prepared from 5 × 10⁶ cells as previously described (38). EMSA was performed with 10 μg of NCE, [γ-³²P]ATP-labeled double-stranded probes, 20 μg of BSA and buffer with 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA, 5% glycerol, 1 mM DTT, 1 mM PMSF, 1 μg of polydeoxyinosinic-polydeoxyctydilic acid, and 2 μg of salmon sperm DNA. The AP-1 probe (Promega) was 5’-CGGTTGATGATGCAGCGC AAGA’-3’, the STAT3 probe (Primm) was 5’-GTCAGCATTTCCGTA AATCG-3’, and the ying yan 1 (YY1) binding site (Promega) was 5’-CTGCAATACGCCATTTTGGCAAGCATTGAA-3’. The radiolabeled double-stranded oligonucleotide corresponding to the YY1 binding site located within the Moloney murine leukemia virus promoter (40) was added as a loading control to the binding mixture together with the AP-1 probe. For supershift analysis, specific Abs (1 μg) against AP-1 members were added to the reaction mixture and incubated for 30 min at room temperature. The mixture was then run at 150 V for 2 h in 5% polyacrylamide gel and 0.5 M Tris-borate and 2 mM EDTA (pH 8.0); the gel was dried at 80°C for 1 h in a gel drier apparatus, and the bands were revealed by overnight autoradiography at ~80°C.

Transient transfections of vectors encoding either urokinase-type plasminogen activator (uPA) promoter regions or HIV LTR of different HIV subtypes

Plasmids containing luciferase were transfected in U1-CRI cells as previously described (9). Briefly, 10⁶ cells were washed twice in PBS and were transiently transfected using a hypotonic DEAE-dextran solution. A total of 3 μg of plasmid was transfected; 4 h later, cells were counted by the trypan blue dye exclusion method, resuspended at 2 × 10⁵ cells/ml, and stimulated with IL-6 in the presence or in the absence of PTX-B (1 nM). In addition, 0.5 μg of the human growth hormone (hGH) plasmid was co-transfected to evaluate the relative efficiency of transfection (hGH levels in culture supernatants were measured 40 h after transfection using an immunofluorometric assay with a sensitivity of 100 pg/ml; TOSOH). Relative luciferase expression (relative luciferase units (RLU)) was measured 40 h after stimulation as described by the manufacturer (Promega), and the results are expressed as the ratio among RLU and micrograms of protein measured in cell lysates (to account for different efficiencies of cell lysis) and levels of hGH in culture supernatants (to account for different efficiencies of transfection). The average protein concentration of 10⁵ cells lysed in 50 μl of lysis buffer was 2 μg/μl, whereas the level of hGH in culture supernatants was ~1.5 ng/ml.

Detection of CXCL8/IL-8 and CCL2/MCP-1

Chemokine secretion in the cell culture supernatants was measured by using commercially available ELISA kits from R&D Systems (detection limit, 10 pg/ml) following the manufacturer’s instructions.

Statistical analysis

Statistical analysis was performed using Student’s t test; a value of p ≤ 0.05 was considered significant.

Results

Differential inhibitory effects of PTX-B on cytokine-induced HIV expression in U1 cells

U1 cells are in a constitutive state of relative latency, testing negative in terms of supernatant-associated RT activity (41). As previously reported in part (9), PTX-B did not induce viral expression in unstimulated U1 cells, whereas it inhibited virus expression induced by a number of cytokines, although with different efficacy (Fig. 1). The strongest anti-HIV activity of PTX-B was observed in IL-6-stimulated U1 cells, although different degrees of suppression were also observed in cells stimulated with TNF-α (9), IL-1β, or...
IFN-γ or after costimulation with GM-CSF and LPS, a combination of stimuli that induces virus production (42) largely because of the endogenous release of IL-1β (43). No interference with either cell viability or proliferation, as indicated by [3H]thymidine uptake was observed in PTX-B-stimulated cells vs their respective controls (data not shown). Because the strongest inhibitory effect of PTX-B was exerted on IL-6-induced HIV expression, we have investigated its mechanism of action in the presence of this cytokine.

**Generation of an IL-6 hyper-responsive clone of U1 cells, U1-CR1**

To better explore the mechanisms underlying PTX-B-mediated abrogation of IL-6-induced HIV expression, U1 cells were seeded in 96-well plates by limiting dilution (0.2 cells/well), and the resulting cell clones were expanded and selected for expressing high levels of IL-6R (95% of positive cells with a mean fluorescence intensity (MFI) of 24.5 vs 4.5 for isotype-stained cells). One of these U1 cell clones, U1-CR1, was further characterized. U1-CR1 cells were comparable to the parental U1 cells in terms of pattern of induction of HIV expression after PMA (data not shown) or TNF-α-stimulation (Fig. 2A). When both parental U1 and U1-CR1 cells were transiently transfected with LTR-luciferase constructs of different HIV-1 clades (A, B, and C) and then stimulated with TNF-α, they showed similar profiles of LTR activation in response to TNF-α (ranging from 5- to 11-fold, with clade C showing the highest levels of induction; Fig. 2B), probably as a consequence of the presence of three rather than two NF-κB binding sites, as reported previously (44). In contrast, U1-CR1 cells were significantly and reproducibly (n = 5) more responsive to IL-6 stimulation than U1 cells (Fig. 2C). Based on these observations, U1-CR1 cells were also used to investigate the modalities of PTX-B interference with IL-6-induced up-regulation of virus production.

**PTX-B does not cause down-regulation of the IL-6R from the cell surface**

To investigate whether the profound inhibition of IL-6-dependent up-regulation of HIV expression in these cells caused by PTX-B was linked to the down-regulation of the cytokine receptor from the cell surface, U1-CR1 cells were stained at different times after incubation with PTX-B by Fluorokine-labeled IL-6. More than 95% of unstimulated U1-CR1 cells expressed IL-6R (MFI, 23.9), and PTX-B did not modify either the percentage of IL-6R+ cells or the average levels of receptor expression per cell (MFI, 22.8; Fig. 3). IL-6-stimulated U1-CR1 cells showed lower levels of IL-6R+ cells compared with unstimulated cells (Fig. 3), whereas PTX-B modified neither the percentage of IL-6R+ cells nor the levels of expression of IL-6R on a per cell basis (MFI, 22.5 vs 20.9 in PTX-B-stimulated vs unstimulated cells, respectively; Fig. 3). Therefore, the strong inhibition exerted by PTX-B on IL-6-induced HIV expression in U1 cells was not caused by a decreased availability of IL-6R from the cell surface.

**PTX-B inhibits IL-6-induced activation of AP-1, but not of STAT3**

As previously demonstrated in parental U1 cells (12), IL-6 did not cause activation of either NF-κB or c-EBP/NF-IL-6 in U1-CR1 cells (data not shown), but it promptly activated STAT3 (Fig. 4A, lane 2), thereby inducing its binding to DNA (Fig. 4B, lanes 4–7). Both STAT3 activation, as measured by Western blot detection of the phosphorylated form (Fig. 4A, lane 2 vs lane 1), and DNA binding (Fig. 4B, lanes 4 and 6 vs lane 1) were clearly induced upon IL-6 stimulation, but not by PTX-B (Fig. 4A, lane 3 vs lane 1; Fig. 4B, lanes 2 and 3 vs lane 1). Of note, PTX-B did not inhibit IL-6-induced STAT3 activation and DNA binding (Fig. 4A, lane 4, and Fig. 4B, lanes 5 and 7), indicating that the profound inhibitory effect on IL-6-induced HIV expression was not due either to an interference with STAT3 activation induced by the cytokine or to a generalized inhibitory effect on IL-6-mediated cell signaling. IL-6 stimulation in the presence or the absence of PTX-B did not modulate the constitutive levels of STAT1 activation and binding (Fig. 4B). In addition, PTX-B did not modulate STAT1 activation and DNA binding in IFN-γ-stimulated U1-CR1 cells (data not shown). It is likely that STAT3 activation induced by IL-6 leads to heterodimerization with constitutively activated STAT1 (middle complex), as previously described (45).
A second family of transcription factors known to be induced upon IL-6 stimulation in different cell types, including monocytic cells, is the dimeric transcription factor AP-1 (46, 47) that has been previously associated with increased transcription and expression of HIV in different cell systems (48), including U1 cells (38, 49). Based on our preliminary experiments (C. Rizzi, unpublished observation) as well as published evidence (38, 49), AP-1 binding to DNA becomes clearly evident 20 h after IL-6 stimulation. Therefore, this time point was chosen to study the potential modulation of PTX-B on this transcription factor by EMSA. Both unstimulated and IL-6-stimulated U1-CR1 cells showed two major AP-1-DNA complexes, as visualized by EMSA, suggesting the presence of both AP-1 homo- and heterodimers (Fig. 5, lane 1 vs lane 3, respectively). The constitutive AP-1 binding activity in unstimulated U1-CR1 cells revealed the presence of all members of Jun and Fos families and of ATF2, as detected by EMSA, followed by Ab-mediated supershift analysis (data not shown), in agreement with a previous report (38). IL-6 stimulation increased binding of AP-1 to its consensus oligonucleotide, and the involvement of individual members of the Jun/Fos families was demonstrated via recognition by specific Ab. Ab-mediated supershift analysis revealed two distinct events, probably depending on whether the Ab directly involved the binding site of the subunit to the consensus DNA. In this case, disappearance or decreased intensity of the bands was visualized (as in the case of c-Jun, JunB, FosB, Fra1, and ATF2; Fig. 5). If Ab binding did not disturb the interaction between the transcription factor and its target DNA, a supershift of the bands was observed (as for JunD, c-Fos, and Fra2; Fig. 5). These results together suggest that all members of the Jun and Fos families as well as ATF2 were present in IL-6-stimulated cells. PTX-B inhibited the AP-1 DNA binding capacity in both unstimulated (Fig. 5, lane 2 vs lane 1) and IL-6-stimulated U1-CR1 cells (Fig. 5, lane 4 vs lane 3). In addition to this inhibitory effect, PTX-B modified the composition of AP-1. Indeed, the bands corresponding to FosB, Fra2, and ATF2 completely disappeared in the presence of their respective Ab (Fig. 5, lanes 14, 18, and 20, respectively), suggesting the possibility that these subunits were relatively overexpressed in the presence of PTX-B. Therefore, the overall decrease in AP-1 binding activity caused by PTX-B was apparently associated with a relative increase in FosB, Fra2, and ATF2 elements.

PTX-B inhibits basal and IL-6-induced AP-1-dependent transcription of the uPA gene in U1-CR1 cells

To investigate whether a functional relationship existed between the inhibitory effect of PTX-B on IL-6-induced HIV expression and AP-1 activation (coupled with a modification of the composition of AP-1 complex), U1-CR1 cells were transfected with a construct containing the upstream regions of the uPA gene, with both specificity protein 1 (Sp1) and AP-1 binding sites (50, 51). Baseline luciferase expression from U1-CR1 cells transfected with either pGL2 (empty vector) or a uPA construct containing the −86 minimal promoter (−86/+320 region, endowed with multiple Sp1 binding sites) (50, 51) was not modified by stimulation with PTX-B, IL-6, or their combination (Fig. 6A). Baseline transcription of the E-86 uPA construct (consisting of the −1977/−1880 enhancer region cloned immediately upstream of the −86 region and containing both Sp1 and AP-1 binding sites) (50, 51) was higher than that observed in −86 transfected cells (Fig. 6A). This observation suggests that the basal level of AP-1 detected in unstimulated U1-CR1 cells may drive gene transcription, whereas IL-6 stimulated and PTX-B inhibited AP-1-dependent E-86-driven transcription (Fig. 6A).

PTX-B inhibits HIV LTR activation in the presence of functional AP-1 binding sites

Having established that AP-1 was transcriptionally active in U1-CR1 cells, we next explored the capacity of PTX-B to interfere with HIV LTR-driven transcription using constructs obtained from different HIV subtypes previously characterized for the presence of AP-1 binding sites (52). Transcription from the LTR of HIV subtypes B and D (lacking functional AP1 binding sites) was up-regulated upon IL-6 stimulation, but was not inhibited by PTX-B (Fig. 6B). Transcription from the LTRs from subtypes C and E (containing one AP-1 binding site) and A and F (containing two AP-1 binding sites) was also up-regulated by IL-6, but PTX-B inhibited luciferase expression from these constructs in both unstimulated and IL-6-stimulated conditions (Fig. 6B). Thus, although IL-6 was found to activate the HIV LTR of subtypes B and D independently from the presence of functional AP-1 binding sites, PTX-B inhibitory effects were tightly associated with the presence of functional AP-1 binding sites in U1-CR1 cells.

PTX-B prevents HIV production and CXCL8/IL-8 and CCL2/MCP-1 secretion in IL-6-stimulated U1-CR1 cells

As demonstrated for parental U1 cells (Fig. 1), PTX-B virtually abolished IL-6-induced HIV expression in U1-CR1 cells (Fig. 7A). To correlate this strong inhibitory effect to that of other cellular
genes, we investigated PTX-B’s capacity to interfere with chemokines such as CXCL8/IL-8 and CCL2/MCP-1, which are transcriptionally regulated by AP-1 (53, 54) and can up-regulate HIV replication both in vitro (16, 55–57) and in vivo (58). Neither chemokine was detectable in the culture supernatants of either unstimulated or PTX-B-stimulated U1 (data not shown) and U1-CR1 (Fig. 7A) cells. In contrast, chemokine secretion was promptly induced after IL-6 stimulation, reaching a peak at 24 h (CXCL8; □) and 48 h (CCL2; ▲), respectively, and decreasing thereafter (Fig. 7B). PTX-B abrogated their secretion (Fig. 7B) with potency and concentration dependency comparable to its anti-HIV effects (Fig. 7C). Ab-mediated neutralization of CCL2 and CXCL8 did not modify HIV expression in either unstimulated or IL-6-stimulated U1 and U1-CR1 cells, probably because of the absent/low levels of expression of their respective receptors, as determined by FACS analysis (data not shown).

Discussion

PTX and PTX-B were originally shown to block entry of CCR5-dependent (R5) virus in a noncompetitive manner as well as R5-and X4-dependent virus replication by additional postentry mechanisms in both primary cells and cell lines (3, 9, 10). In this model, chemokine secretion was promptly induced after IL-6 stimulation, reaching a peak at 24 h (CXCL8; □) and 48 h (CCL2; ▲), respectively, and decreasing thereafter (Fig. 7B). PTX-B abrogated their secretion (Fig. 7B) with potency and concentration dependency comparable to its anti-HIV effects (Fig. 7C). Ab-mediated neutralization of CCL2 and CXCL8 did not modify HIV expression in either unstimulated or IL-6-stimulated U1 and U1-CR1 cells, probably because of the absent/low levels of expression of their respective receptors, as determined by FACS analysis (data not shown).
but not c-Jun/c-Fos, heterodimers may exert repressive effects on AP-1-dependent transcription, as observed in the case of uPA (67). Thus, a high abundance of ATF2 could lead to increased formation of c-Jun/ATF2 complexes and inhibition of uPA gene expression, as observed in this study in U1-CR1 cells in the presence of PTX-B.

PTX and PTXB interfere with the HIV life cycle at multiple levels, from inhibiting entry of R5 HIV by a noncompetitive mechanism (3, 10) to additional postentry inhibitory effects (9, 10). Although PTX-B suppression of IL-6-induced HIV expression in both U1 and U1-CR1 cells was almost absolute, inhibition of LTR-driven transcription was restricted to those promoters including AP-1 binding sites, such as those derived from clades A, C, E, and F, whereas it was not observed in cells transfected with clade B and D LTR, devoid of these sites (52). This observation suggests that PTX-B inhibitory effects on HIV expression in U1 and U1-CR1 cells, originally infected with the X4LAI/IIIB subtype B HIV-1 (41), is likely to occur at a posttranscriptional step. In this regard, our original description of the differential features of IL-6- vs TNF-α-induced HIV expression pointed out to a major role of posttranscriptional events in IL-6-mediated up-regulation of viral production from U1 cells (12). Alternatively, PTX-B could down-regulate AP-1 mediated activation of the intragenic gag enhancer (48, 60), previously suggested to be induced by stimuli, such as IL-6, in combination with TNF-α or glucocorticoids (38, 49). In this regard, our findings on the levels of luciferase expression driven by LTRs after IL-6 stimulation add to previous reports on biological differences among the different HIV clades, which are determined in part by differences in the composition of the LTR promoter (52, 77–80). Thus, differences in the composition of transcription factor binding sites may provide each clade with a promoter responding in a unique manner to cellular activation pathways.

AP-1-dependent transcription may lead to the up-regulation of several cytokines (54), including the chemokines CXCL8/IL-8 and CCL2/MCP-1. The secretion of both chemokines is up-regulated after in vitro HIV infection of primary PBMCs and MDM (56, 81) or after IL-6 stimulation of MDM and promonocytic U1 and U937 cells (15). In vivo, increased levels of CXCL8/IL-8 were observed in the lymphoid tissues of HIV+ individuals (16), whereas high levels of CCL2/MCP-1 were observed in the cerebrospinal fluid of AIDS patients with either CMV- or HIV-associated encephalitis (58, 82). Both chemokines were shown to up-regulate HIV replication in primary PBMC and MDM infected in vitro (16, 57) as

FIGURE 6. PTX-B (1 nM) inhibition of uPA promoter and HIV-1 LTR activation is dependent upon the presence of AP-1 DNA binding sites. A, U1-CR1 cells were transfected with plasmids containing different upstream promoter regions of the uPA gene with (E-86) or without (−86) AP-1 DNA binding domains, respectively. Only the E-86 plasmid showed superior baseline levels as well as responsiveness to IL-6 and inhibition by PTX-B. B, Plasmids containing LTRs derived form different HIV-1 subtypes were transfected into U1-CR1 cells, which were then stimulated, or not, with IL-6 (10 ng/ml) in the presence or the absence of PTX-B. The results are expressed as the fold increase in stimulated vs unstimulated cells after normalization of the RLU with micrograms of proteins (control for cell lysis) and levels of hGH (control for efficiency of transfection). IL-6 up-regulated all the LTRs, although with different potencies, whereas PTX-B inhibited both basal and IL-6-induced LTR activities only in those containing functional AP-1 binding sites (i.e., A, C, E, and F). Results are expressed as the mean ± SD of three independent experiments, each run in duplicate (with an intra-assay coefficient of variation <5%).

FIGURE 7. PTX-B inhibits IL-6-induced CXCL8 and CCL2 expression from U1-CR1 cells. U1-CR1 cells were incubated with PTX-B (1 nM) and stimulated after 5 min with IL-6 (10 ng/ml). The levels of chemokine and HIV expression were measured by RT activity every 24 h in the culture supernatants. The results from one representative experiment, tested in duplicate, of six independently performed are shown. A, PTX-B (1 nM) suppresses IL-6-induced HIV expression in U1-CR1 cells. B, Both CXCL8 and CCL2 were undetectable in unstimulated U1-CR1 cells. IL-6-induced secretion of CXCL8 and CCL2 (■ and △) in U1-CR1 cells, and this effect was significantly inhibited by PTX-B (1 nM; □ and ▽). C, Comparable concentration dependencies of PTX-B inhibitory effects on IL-6-induced up-regulation of HIV (by reverse transcriptase activity) and chemokine expression (picograms per milliliter in culture supernatants) in U1-CR1 cells.
well as in PBMCs cultured from HIV-infected individuals (16, 55, 57), although they were not effective in an autocrine fashion in U1 (or U1-CR1) cells, probably because of the absence of low levels of expression of their receptors. Thus, IL-6 triggered the secretion of CXCL8 and CCL2, and the inhibitory effect exerted by PTX-B appears to be regulated by AP-1.

In conclusion, our study provides novel evidence of the importance of IL-6 and IL-6-induced AP-1 in HIV transcription and defines precisely a molecular correlate (i.e., inhibition of AP-1) of the broad spectrum of anti-HIV activities of PTX-B. These results support the hypothesis that PTX-B or related molecules, such as the genetically modified PT-9K/129G (83), which retains all the HIV inhibitory features of PTX-B (9, 26, 84), could represent potent new pharmacological agents against HIV infection, as we have also recently demonstrated in infected lymphoid histiocytoses (85) and hu-PBL SCID mice infected with HIV (86).

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Disclosures

The authors have no financial conflict of interest.

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