Suppression of HIV-1 transcription by β-chemokines RANTES, MIP1-α, and MIP-1β is not mediated by the NFAT-1 enhancer element

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Abstract Soluble factors derived from human CD8+ T-lymphocytes inhibit HIV-1 replication by suppressing transcription from the viral long terminal repeat (LTR), an effect shown to be mediated in part by an NFAT-1 enhancer sequence. We show here that the CD8+ derived β-chemokines, RANTES, MIP1-α, and MIP-1β, known suppressors of HIV-1 replication in human peripheral blood mononuclear cells, can suppress transcription from the HIV-1 LTR in transient transfection assays in cells of the Jurkat (acute T leukemia) line. Surprisingly, the suppression mediated by these β-chemokines persisted in the absence of an intact NFAT-1 element, suggesting that there are at least two classes of HIV-1 suppressor factors — NFAT-1-dependent and NFAT-1-independent factors — produced by CD8+ T-lymphocytes.

Key words: HIV-1; Long terminal repeat; β-Chemokine; Transcription

1. Introduction

CD8+ T-lymphocytes have been shown to produce soluble factor(s) active in suppressing HIV-1 transcription [1–3], though the mechanism of action remains to be elucidated. Copeland and colleagues [4] showed that this soluble factor(s)-mediated suppression of HIV-1 transcription required the presence of an intact NFAT-1 element in the LTR of HIV-1.

Recently, Lusso and colleagues [5] identified several of these CD8+-derived HIV-suppressive factors (HIV-SF) as the β-chemokines RANTES, MIP1-α, and MIP-1β. These chemokines have been shown to exert their inhibitory effects when added exogenously to cultures of HIV-1 infected T-cell clones and primary peripheral blood mononuclear cells (PBMC).

These two lines of evidence led us to investigate whether the observed suppressive effects of RANTES, MIP1-α, and MIP-1β, were effective at the level of transcription, mediated by the HIV–LTR NFAT-1 element.

2. Material and methods

2.1. Cell culture

The Jurkat cell line (acute T-cell leukemia) used in this investigation was obtained from the American Type Tissue Collection (Rockville, MD) and cultured in RPMI-1640 medium (Biofluids Inc., Rockville, MD) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Hyclone Laboratories Inc., Gaithersburg, MD), 2 mM L-glutamine (Quality Biologicals Inc., Gaithersburg, MD), and 100 U/ml penicillin+100 μg/ml streptomycin (Quality Biologicals). All cells were grown at 37°C with 5% carbon dioxide in a humidified incubator.

2.2. Vectors

The HIV–LTR–CAT constructs were a gift of Dr. Gary Nabel (Howard Hughes Medical Institute, University of Michigan) The vector pLTRCAT contains HIV-1 LTR sequences of the BRU strain to position +77 directing chloramphenicol acetyl-transferase (CAT) expression [4]. The mutant HIV-1 LTR vector has the NFAT-1 IL-2 homology region altered by site-directed mutagenesis [6].

2.3. Transfection

Cells were transiently transfected with the various reporter gene constructs by electroporation, as previously described [7]. Briefly, cells were grown to a density of $0.5–1\times10^6$ cells/ml, harvested, washed, and resuspended at 30×10^6 cells/ml in growth medium. Ten micrograms of uncut reporter gene construct along with 10 μg uncut pCMV–β-galactosidase plasmid (Promega) were added to an 0.5 ml of cell suspension in an electroporation cuvette (0.4 cm gap) (Bio-Rad, Hercules, CA) and pulsed at 250 V, 960 μFd. Transfected cells were resuspended in 30 ml complete medium with or without chemokines and incubated at 37°C, 5% carbon dioxide for 2 days prior to harvesting. Expression of CAT was normalized to β-galactosidase activity.

2.4. CAT assays

CAT assays were performed as described previously [7]. Briefly, 10–30 μl of cell extract was added to a reaction mixture containing 35 μl of 1 M Tris-HCl, pH 7.5, 20 μl of 4 mM acetyl coenzyme A (Pharmacia, Piscataway, NJ), 5 μl of [^{14}C]chloramphenicol (0.25 μCi/μl; New England Nuclear, Boston, MA) and dH_20 to 150 μl. After 1–3 h incubation at 37°C, the [^{14}C]chloramphenicol and acetylated derivatives were extracted with 0.5 ml of ethyl acetate, dried, resuspended and applied to a thin layer chromatography sheet (Baker-flex silica gel 1B, J.T. Baker Inc., Phillipsburg, NJ). Separation of acetylated and non-acetylated forms proceeded via a chloroform/methanol (95:5) ascending mobile phase followed by autoradiography. The relative intensity of each signal was measured by densitometric scanning (UVP, San Gabriel, CA) and analysis (NIH Image software). Experiments were performed in duplicate; representative data is presented.

3. Results and discussion

Jurkat cells, transiently transfected with the HIV-1 (BRU strain) LTR–CAT constructs (Fig. 1) were incubated for 48 h following transfection, both in the presence and absence of RANTES (20 ng/ml), MIP1-α (100 ng/ml), and MIP-1β (50 ng/ml). As shown in Fig. 2, reporter gene activity was measured in the transfected Jurkat cells. In this system, the wild-type HIV LTR promoted reporter gene activity at ~6-fold over the background level seen with the promoterless pCAT basic construct. In the presence of the β-chemokines, reporter gene activity from the wild-type HIV–LTR was reduced to nearly one-third of the activity observed in their absence. Disruption of the NFAT-1 enhancer element did not alter the reporter gene activity promoted by the LTR in the absence

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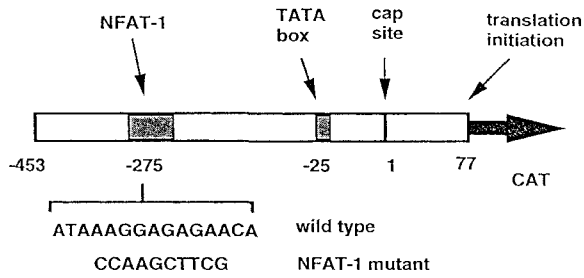

Fig. 1. Structure of the wild-type parent HIV-1 LTR and the NFAT-1 mutant LTR positioned to direct CAT reporter gene expression ([4,6]).

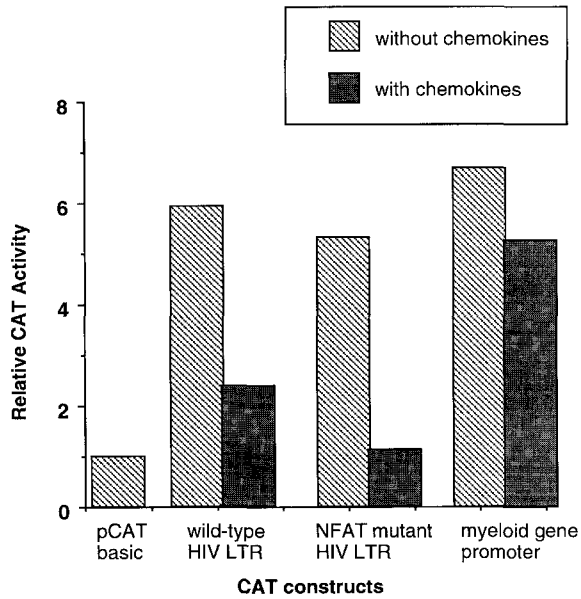

Fig. 2. Comparison of the effects of β-chemokine treatment, RANTES (20 ng/ml), MIP1-α (100 ng/ml), and MIP-1β (50 ng/ml), on HIV-1 LTR–CAT construct reporter gene expression. The irrelevant control is the tandem promoter, exon 1, and intron of the eosinophil-derived neurotoxin gene placed upstream of CAT ([7]). Experiments were done in duplicate; a representative sample is shown.

of β-chemokines (~5.5-fold over background level). Surprisingly, the β-chemokine combination also maintained transcriptional suppression in the absence of an intact NFAT-1 enhancer element, reducing reporter gene activity to near background levels. As an irrelevant promoter control, transcriptionally active elements of the myeloid eosinophil-derived neurotoxin gene [7] were evaluated in this system; only minimal chemokine-mediated suppression was observed.

Our data suggests that the NFAT-1-mediated suppression observed by Copeland and colleagues [4] involves HIV–SFs other than RANTES, MIP1-α, and MIP-1β. Furthermore, our data supports the concept that the β-chemokines interfere with HIV-1 replication by transcriptional suppression, a function that likely coordinates with their role in blocking viral entry at the membrane receptor level [8,9].

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