Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 329 (2005) 706–712
BBRC
www.elsevier.com/locate/ybbrc

HIV-1 Tat modulates T-bet expression and induces Th1 type of immune response

Asavari Kulkarni, Dyavar S. Ravi, Kamini Singh, Shravanti Rampalli, Vrajesh Parekh, Debashis Mitra, Samit Chattopadhyay *

National Center for Cell Science, Pune University Campus, Ganeshkhind, Pune 411 007, India

Received 20 December 2004

Abstract

The HIV-1 transactivator Tat performs various viral and cellular functions. Primarily, it induces processive transcription from the HIV-1 LTR promoter. However, Tat secreted from infected cells is known to activate uninfected lymphocytes through receptors. To further delineate the specific target genes, extracellular Tat was expressed on the cell membrane of stimulator cells and co-cultured with human PBMCs. Along with induced $CD4^+$ T cell proliferation and IFN-γ secretion, there was strong upregulation of T-bet expression which is majorly implicated in generating T_H1 type of immune response. To further delineate the effect of extracellular Tat on HIV replication, both p24 analysis and in vivo GFP expression were performed. There was a significant inhibition of HIV-1 replication in human CEM-GFP cell line and hPBMCs. Thus, for the first time we report that apart from its transactivation activity, extracellular Tat acts as a costimulatory molecule that affects viral replication by modulating host immune response through induction of T-bet expression and IFN-γ secretion.

Keywords: HIV-1; Tat; T cell proliferation; T-bet; IFN-γ; TCR; HIV-1 replication

The human immunodeficiency virus (HIV-1) has been linked with the acquired immunodeficiency syndrome (AIDS) [1]. The HIV-1 viral genome encodes three structural genes (*gag*, *pol*, and *env*), three regulatory genes (*tat*, *nef*, and *rev*), and three accessory genes (*vpr*, *vpu*, and *vif*), all in overlapping frames. These genes are flanked by a 5′ and a 3′ long terminal repeats (LTR) region that contains enhancer and promoter elements essential for proviral transcription and replication [2].

The HIV-1 transactivator Tat is a 102 amino acids long RNA binding protein that facilitates HIV-1 transcription and replication [3]. Tat is known to bind various cellular factors like Cdk 9, cyclin T1, TFIIH, and core Pol II, and promote LTR mediated transcription [4]. In addition to transactivation, it regulates other cellular and viral genes [5], thereby activating and making uninfected and quiescent T cells more susceptible to HIV-1 infection [6]. Tat also induces IL-10 production from human PBMCs, which is dependent on the activation of PKC, NF-κB, and MAP kinases-ERK1/2 [7].

Amongst lymphocyte populations, upon antigenic stimulation, naive $CD4^+$ T helper cells differentiate into two subsets, T_H1 and T_H2, exhibiting distinct functions and cytokine profiles. T-bet, a T_H1-specific T box transcription factor, controls the expression of T_H1 cytokines and initiates T_H1 lineage development from naive T_H cells. This is achieved by simultaneous activation of T_H1 and repression of T_H2 genetic programs. In this respect IFN-γ, a hallmark T_H1 cytokine, is induced by T-bet through its promoter that harbors T-bet response elements [8]. HIV-1 favors a Th1 type of immune response during the initial stages of infection, which is predominantly driven by Tat [9].

* Corresponding author. Fax: +91 20 569 2259.
E-mail address: samit@nccs.res.in (S. Chattopadhyay).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.02.042

IFNs are known to block the replication of HIV-1 at a later stage of viral replication that leads to a decrease in viral protein stability and dysregulating polyprotein processing [10]. A concurrent increase in the production of IFN-γ during infection results in inhibition of HIV-1 replication. This is achieved by disrupting Tat interaction with other cellular factors thereby establishing HIV-1 latency during later stage of infection [11]. Thus, the regulation of T-bet expression by Tat is critical to understand its role in switching immune response during HIV-1 pathogenesis.

Chen et al. reported the use of LAMP-1 (Lysosomal Associated Membrane Protein) (CD107b), a major lysosomal membrane glycoprotein [12] for the expression of chimeric proteins like gp120 and CD4 on the cell surface [13]. To further understand the role of surface associated Tat in modulating immune response, we have generated a fusion construct of Tat–LAMP. Here we show that the chimeric Tat protein expressed on the surface of heterologous cells activates and induces proliferation of co-cultured human PBMCs. This was found to be dependent on the engagement of CD3/TCR cross-linking. Moreover, we show that the surface bound Tat elicits a T_H1 type of response inducing T-bet expression by increasing IFN-γ secretion. Further investigation of its role in the context of HIV-1 revealed that extracellular Tat significantly inhibits HIV-1 replication Thus, we propose that extracellular Tat induces IFN-γ by triggering T-bet expression and finally impedes HIV-1 replication.

Materials and methods

DNA constructs. For expression of membrane bound Tat, HIV-1_{HXBIII} Tat was cloned in pcDNA 3.1 [14]. LAMP-1 was originally cloned from the mouse embryonic cell line 3T3 into phagemid λgt11. LAMP was excised using *Bam*HI–*Xho*I and subcloned into the same sites of pcDNA 3.1 (Invitrogen). For amplification of Tat gene, PCR was performed using 5′-CTAGAAGCTTATGGAGCCAGTAGATC CTAG (forward) and 5′-CGTCGGATCCCCTTCGGGCCTGTC GGGTCC (reverse) primers. A 0.3 kb fragment was first cloned into pGEMT-Easy (Promega) and further subcloned upstream of LAMP in pcDNA construct using *Nhe*I and *Bam*HI restriction enzyme sites. This in-frame Tat–LAMP clone was then used for expression of membrane bound Tat. We have also generated an out-of-frame construct of Tat–LAMP, which served as a negative control. A single base deletion reverse primer was used during PCR and identical RE sites were used for cloning. All the constructs were confirmed by sequencing.

RT-PCR analysis. For RT-PCRs of human T-bet, the total RNA was extracted using Trizol reagent (Gibco) and quantitated by UV spectrophotometer. Five micrograms of mRNA was taken for cDNA preparation by MMLV-RT, in a 20 μl reaction (5 μg RNA, 1× first strand buffer, 1 mM DTT, 0.125 mM dNTPs, random primers, and RNase out) (Gibco) at 37 °C for 1 h. The cDNA template was used for PCR using the primers H-Tbet1-5′-TGCGGAGACATGCTGACG; H-Tbet2 5′-ATCTCTGTCTGGTGCTGATTA at 57 °C annealing temperature.

Cell lines and transfections. Human embryonic kidney (HEK)-derived 293 cells obtained from the NCCS repository were cultured in DMEM supplemented with 10% FCS (Gibco-BRL). CEM-GFP cells were grown in RPMI 1640 (Invitrogen) supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. Transfections were done with 1 μg plasmid DNA constructs as indicated, using Lipofectamine 2000 (Gibco-BRL), following the manufacturer's instructions.

Luciferase assay. Luciferase assays were performed using a single luciferase assay reporter system (Promega). After 48 h of transfection, the cells were washed with 1× PBS, lysed in 200 μl reporter lysis buffer (Promega), and subjected to two cycles of freeze thawing for complete lysis. The samples were then centrifuged at 10,000 rpm for 15 min and an equal amount of protein (75 μg) was used for the assay. Luciferase activity was measured using Fluoroskan Ascent FL Luminometer (Labsystems).

Cytokine ELISAs. For analyzing hIFN-γ and hIL-4 cytokines in the supernatants of the co-culture assay, cytokine specific sandwich enzyme-linked-immunosorbent assay (ELISA) kits were used (BD, Pharmingen). For assaying IFN-γ secretion from infected cells, the respective cells were lysed in 0.01% Triton X, centrifuged at 1000 rpm for 10 min, and the supernatants were subjected to ELISA.

Confocal microscopy. HEK293 cells were transfected and processed for confocal analysis [15]. The antibodies used were 1/100 diluted mouse anti-Tat (Autogen Bioclear), 1 μg/ml rat anti-LAMP (BD, Pharmingen), 1/100 anti-mouse (Sigma), and 1/50 anti-rat FITC-conjugated IgG (Dako). The slides were observed using a Confocal Laser microscope (LSN 510, version 2.01; Zeiss, Thornwood, NY).

Western blotting. Cell pellet was lysed in 150 μl Dignam buffer C (20 mM Hepes, pH 7.9, 25% glycerol, 0.42 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, and protease inhibitor cocktail) for Tat protein extraction and Triton X lysis buffer (1% Triton X, 50 mM Tris–Cl, pH 7.5, 1 mM EDTA, 150 mM NaCl, and protease inhibitor cocktail), for isolation of cell surface proteins LAMP and Tat–LAMP. Whole cell proteins were resolved in SDS–PAGE, transferred onto a PVDF membrane (Amersham), and further processed for Western blot analysis.

Isolation and infection of human PBMCs. Human blood was collected from healthy donors in a heparinized tube and subjected to Ficoll–Paque (Amersham) centrifugation. The interphase cells were taken and cell counting was done. 10^6 cells were cultured for 24 h in RPMI and 10% FCS with 20 U recombinant hIL-2 (Stem Cell) and 1 μg/ml PHA-M (Amersham). For infection, the cells were centrifuged twice at 1000 rpm for 5 min and resuspended in 0.5 ml RPMI and 10% FCS, with 2 μg/ml polybrene. Twenty-five nanograms p24 units of HIV-$1_{NL4.3}$ strain (25 μl) was then added and incubated for 4 h with intermittent mixing. The levels of p24 were monitored on the 3rd, 4th, and 5th day, and quantified, by comparing with a p24 standard graph of recombinant p24.

Co-culture assays of infected and uninfected hPBMCs. For the hPBMC proliferation assay, HEK293 cells were transfected with either Tat–LAMP or control plasmids and then selected for 10 days using 400 μg/ml G418 (USB). These stimulator cells were fixed in 2.5 μg/ml mitomycin C (Roche) for 25 min and washed thrice in DMEM with 10% FCS. Purified, uninfected PBMCs (1×10^5) were then co-cultured with (1×10^4) stimulator cells for 72 h in a 96-well plate (Nunc). For T cell activation, 1 μg/ml human monoclonal anti-CD3 antibody (BD, Pharmingen) was added. After 64 h, 1 μCi [^{3}H]thymidine (BRIT) was added and pulsed for another 8 h. The cells were then harvested by a semi-automatic Cell harvester (Skatron) and thymidine incorporation was measured. Each set was performed in triplicate. For p24 analysis, infected CEM-GFP cells and/or hPBMCs were centrifuged at 1000 rpm for 5 min. The stimulator cells (1×10^4) were added in a 96-well plate to the infected cells (0.25×10^5) and incubated at 37 °C for 48 h (with anti-CD3 for hPBMC assay). The lysed supernatants were assayed for p24 ELISA. This was done following the manufacturer's protocol (HIV-1 p24 ELISA kit, NEN Perkin-Elmer Life Sciences).