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HIV-1-mediated syncytium formation promotes cell-to-cell transfer of Tax protein and HTLV-I gene expression

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Abstract

An important increase in luciferase activity was detected following co-culture of Jurkat T cells transiently transfected with an HTLV-I-LTR-driven reporter construct with HIV-1- and HTLV-I-infected cells. Production of infectious HTLV-I and expression of the HTLV-I envelope were not required for this HIV-1-dependent induction while it was severely hampered by anti-gp120 and anti-CD4 antibodies. The HTLV-I Tax protein and the TRE1 repeats were found to be necessary for the HIV-1-mediated enhancement of HTLV-I LTR activity in the co-culture assay. As these results suggested triple fusion events involving all three cell types and the intracellular transfer of Tax, we labelled each cell line with a distinct fluorescent probe. Through confocal microscopy, a number of resulting syncytia and cell clusters were indeed observed to be positive for all three probes. We are proposing a model in which HIV-1-mediated syncytium formation between HIV-1- and HTLV-I-infected cells and uninfected T cells forms a "bridge" or "tunnel" through which Tax from the HTLV-I-infected cells can diffuse and activate HTLV-I-LTR transcription.

Keywords: HIV-1; HTLV-I; Syncytium; T cell

1. Introduction

Human T cell leukemia virus type I (HTLV-I) is a deltaretrovirus and the etiologic agent of Adult T cell Leukemia and HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis (Gessain and Gout, 1992; Greenberg, 1995; Poiesz et al., 1980; Weiss, 1992). As for human immunodeficiency virus type 1 (HIV-1), lymphoid organs are believed to be a major reservoir for HTLV-I in vivo, followed by the peripheral blood mononuclear cells (Kazanji et al., 2000; Takenouchi et al., 1999). The provirus is mainly found in $CD4^+$ T lymphocytes in vivo, although up to a quarter of the provirus load may be carried by $CD8^+$ T cells (Bangham, 2003). Infectivity of cell-free HTLV-I virions tends to be very weak, and the virus appears to be transmitted most efficiently by the cell-to-cell route (Markham et al., 1983). Even though several findings suggest a continuous expression of viral proteins in a portion of HTLV-I-infected cells, at least in symptomatic individuals (Jacobson et al., 1990; Kinoshita et al., 1989; Niewiesk et al., 1995), in vivo most of the infected cells have long been believed to be latent (Kazanji et al., 2000; Richardson et al., 1997; Wodarz et al., 1999), as evidenced by the presence of an importantly low number of $CD4^+$ T cells producing virions as compared to those bearing proviral DNA in the blood. During the so-called "latency" period, the remarkably high proviral load found in HTLV-I infection, i.e. typically 0.1–10% of peripheral blood mononuclear cells (PBMCs) carry the provirus (Gessain et al., 1990; Richardson et al., 1990; Shinzato et al., 1991; Wattel et al., 1992), seems to be maintained largely by mitotic division of provirus-containing cells. Viral gene expression can nonetheless be detected from ex vivo cultured PBMCs harvested from infected individuals after a few hours (Hanon et al., 2000a).

The HTLV-I genome carries the *gag*, *pol* and *env* genes, which are characteristic of all retroviruses, and also encodes a number of regulatory proteins such as Tax and Rex. The Tax gene encodes the main target antigen eliciting HTLV-I-specific CTL response. The Tax protein is an early expressed HTLV-I protein that acts as a potent stimulator of viral transcription

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(Brady et al., 1987; Sodroski et al., 1984) and displays an oncogenic potential (Grassmann et al., 1992, 1989). Tax does not bind DNA directly but rather associates via protein–protein interactions with cellular proteins bound to the viral promoter (Giam and Xu, 1989; Marriott et al., 1989). Each viral promoter contains three copies of a 21-bp imperfect repeat called Tax-responsive element-1 (TRE-1). Cellular proteins such as HEB, TREB1, TREB2, CRE binding protein (CREB) and TF1 have been demonstrated to bind TRE1 in vitro (Beraud et al., 1991; Tan et al., 1989; Tillmann et al., 1994; Tillmann and Wigdahl, 1994). Previous studies have demonstrated that these sequences are crucial for both basal transcription and Tax-related activation of viral transcription (Barnhart et al., 1997; Giam and Xu, 1989).

Infection with HTLV-I occurs in 5–10% of HIV-1-infected individuals in many urban areas. This proportion is at least 100–500 times greater than in the general population (i.e. less that 0.05% among US blood donors) (Khabbaz et al., 1992). However, the clinical significance of HIV-1 and HTLV-I co-infection is still unclear. Co-infected individuals appear to be at higher risk for the development of HTLV-I-associated diseases (Wiley et al., 1989). Moreover, expression of HTLV-I-encoded *tax/rex* at the mRNA and protein levels are upregulated in PBMCs isolated from patients co-infected with HTLV-I and HIV-1 (Beilke et al., 1998; Kubota et al., 1998). Many studies have already been performed to address the possible modulatory role of HIV-1 on HTLV-I gene expression. For example, superinfecting HTLV-I-transformed immortalized cells such as MT-2 and C8166-45 with infectious HIV-1 particles resulted in an increase in HTLV-I protein expression while the HIV-1 Rev protein was found to augment HTLV-I production (Beilke et al., 1998; De Rossi et al., 1991; Kubota et al., 1998).

In the present study, we were interested in defining whether co-culture between HIV-1- and HTLV-I-infected cells can affect HTLV-I LTR-mediated gene expression. In order to evaluate such a possible viral interplay, we tested an in vitro experimental model system made of three different cell lines that includes chronically HIV-1-infected cells, HTLV-I-transformed cells and Jurkat cells transiently transfected with an HTLV-I LTR reporter construct. We demonstrate here that the Tax protein can diffuse into Jurkat cells through HIV-1-mediated cell fusion and potently mediate HTLV-I LTR-driven transcription. Our results thus suggest that, in persons dually infected with HTLV-I and HIV-1, HIV-1-induced cell-to-cell fusion might function as a biological "bridge" or "tunnel" through which Tax protein from chronically HTLV-I-infected cells could diffuse into other T cells infected and therein activate latent HTLV-I provirus.

2. Materials and methods

2.1. Cell lines

The human T lymphoid cell line Jurkat (clone E6.1) was used in this study (Weiss and Stobo, 1984). MT-2 is a HTLV-I-infected T cell line that secretes infectious HTLV-I particles (Haertle et al., 1988; Harada et al., 1985). C8166-45 is a T cell line containing an HTLV-I genome that only expresses the *tax* gene (Sodroski et al., 1984). H9/HIV-1_{IIIB} cells were derived from the H9 cell line chronically infected with HIV-1 strain IIIB (Mann et al., 1989). A2.01/2.0 is derived from the CD4-negative T cell line A2.01 and stably expresses envelope proteins from HIV-1 strain SF33 (Moir and Poulin, 1996). JPX-9 is a Jurkat derivative stably transfected with a plasmid carrying the p40tax gene under the control of the inducible metallothionein promoter (kindly provided by Dr. Nakamura, Tohoku University, Sendai, Japan) (Nagata et al., 1989).

2.2. Plasmids.

The pHTLV-LUC vector was kindly provided by Dr. W.C. Greene (Gladstone Institute of Virology and Immunology, San Francisco, CA) and contains the HTLV-I LTR cloned upstream of the luciferase reporter gene in the pGL2-Basic vector (Promega, Madison, WI) (Geleziunas et al., 1998). The pNF-κB-LUC vector was obtained from Stratagene (La Jolla, CA) and contains multiple NF-κB-binding sites. The pNFAT-LUC construct contains the minimal IL-2 promoter with three tandem copies of the NFAT1-binding site (a kind gift from Dr. G. Crabtree, Howard Hughes Medical Institute, Stanford, CA) (Timmerman et al., 1996). The pU3R/CAT and p21PMD/11TCAT mutants were kindly provided by Dr. S.J. Marriott (Baylor College of Medicine, Houston, TX). The pU3R/CAT plasmid includes the CAT reporter gene controlled by a wild type U3/R region from the HTLV-I-LTR region, while p21PMD/11TCAT contains a mutated U3/R region resulting from a C-to-T change in the position 11 of all the three 21-bp TRE1 sequences (Barnhart et al., 1997). pU3R/CAT and p21PMD/11TCAT were digested with *Xho*I and *Hind*III and the fragment containing the HTLV-I promoter was cloned into pGL3basic (Promega) at the same restriction sites to generate pGL3-U3R (called U3R) and pGL3-21PMD (called 21PMD).

2.3. Antibodies and reagents

The anti-gp46 antibody LAT-27 was provided by Dr. Tanaka (Ryukyu University, Nichihara, Okinawa, Japan) (Okuma et al., 1999). The monoclonal anti-gp120 antibody 0.5β maps to amino acids 308–331 of gp120 and was previously shown to inhibit virus-mediated syncytium formation and infection with cell-free HIV-1_{IIIB} (Matsushita et al., 1988). Monoclonal anti-CD4 antibody SIM.2 recognizes a different epitope from Leu 3a and blocks HIV-1-mediated syncytium formation (McCallus et al., 1992). The fluorescent probes DiI (1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbocyanine perchlorate; excitation/emission [ex/em] 550/560 nm), DiO (3,3′-dioctadecyloxaocarbocyanine perchlorate; ex/em 484/501 nm) and CMAC (7-amino-4-chloromethylcoumarin; ex/em 354/469 nm) were all purchased from Molecular Probes (Eugene, OR).

2.4. DEAE-Dextran transfection, co-culture experiments and luciferase assay

Transient transfections were done using the DEAE-Dextran method as previously described (Barbeau et al., 1997).