Virology 508 (2017) 7–17

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

RNA stability regulates human T cell leukemia virus type 1 gene expression in chronically-infected CD4 T cells

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ARTICLE INFO

Keywords:
HTLV-1
Latency
RNA stability
Retroviruses
Arginosuccinate lyase

ABSTRACT

Regulation of expression of HTLV-1 gene products from integrated proviruses plays an important role in HTLV-1-associated disease pathogenesis. Previous studies have shown that T cell receptor (TCR)- and phorbol ester (PMA) stimulation of chronically infected CD4 T cells increases the expression of integrated HTLV-1 proviruses in latently infected cells, however the mechanism remains unknown. Analysis of HTLV-1 RNA and protein species following PMA treatment of the latently HTLV-1-infected, FS and SP T cell lines demonstrated rapid induction of *tax/rex* mRNA. This rapid increase in *tax/rex* mRNA was associated with markedly enhanced *tax/rex* mRNA stability while the stability of unspliced or singly spliced HTLV-1 RNAs did not increase. *Tax/rex* mRNA in the HTLV-1 constitutively expressing cell lines exhibited high basal stability even without PMA treatment. Our data support a model whereby T cell activation leads to increased HTLV-1 gene expression at least in part through increased *tax/rex* mRNA stability.

1. Introduction

The human T cell leukemia virus type 1 (HTLV-1) was the first discovered human retrovirus and is the causative agent of adult T cell leukemia/lymphoma (ATL) and HTLV-associated myelopathy/ tropical spastic paraparesis (HAM/TSP) (Gallo, 2005; Yoshida, 2005). HTLV-1 infects an estimated 5–10 million people worldwide (Gessain and Cassar, 2012; Goncalves et al., 2010). Approximately 2–6% of HTLV-1-infected individuals develop ATL, a devastating malignancy of CD4 T cells, generally after a prolonged period (20–60 years) of clinical latency (reviewed in Iwanaga et al. (2012), Yasunaga and Matsuoka (2007)). HAM/TSP has been estimated to occur in 5–10% of infected patients with recent studies suggesting neurological symptoms can be seen in > 30% of patients over an eight year follow-up (Tanajura et al., 2015). HAM/TSP is associated with expansions of both infected CD4 T cells and activated CD8 T cells (reviewed in Araya et al. (2011), Nagai and Osame (2003)). The pathogenesis of both ATL and HAM/TSP remains unclear, particularly with respect to the factors that determine whether infected individuals develop disease, the determinants of the disease phenotype, and the events that occur during the clinically latent period.

HTLV-1 infects primarily CD4 T cells. As with other retroviruses, the HTLV-1 genome integrates into host DNA. Following integration, infected cells can undergo a productive infection with generation of progeny virus, or can enter a state of low or no virion production (i.e. viral latency) (reviewed in Carpentier et al. (2015)). The existence and nature of HTLV-1 latency has been somewhat controversial. The continued induction of high levels of anti-HTLV-1 cytotoxic T lymphocyte (CTL) response, particularly directed against the HTLV-1 Tax transactivator protein as well as the HBZ protein, is evidence that there is significant production of viral antigens even during clinically quiescent periods. The strong CTL response has been argued to be responsible for the lack of evidence of viral gene expression in blood (Asquith et al., 2000). On the other hand, at any given time, the number of cells bearing the HTLV-1 genome exceeds the number of cells in which viral RNA species and viral proteins can be detected (Gessain et al., 1991; Hollsberg, 1999). Even a brief period of ex vivo culture is sufficient to detect markedly increased HTLV-1 expression in

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http://dx.doi.org/10.1016/j.virol.2017.04.029
Received 6 January 2017; Received in revised form 25 April 2017; Accepted 26 April 2017
Available online 04 May 2017
0042-6822/

cells (Hanon et al., 2000). By analogy with HIV and other retroviruses, these data suggest that a significant number of HTLV-1 infected cells are latently infected in vivo. Furthermore, considerable DNA sequencing data show that the major route of HTLV-1 expansion in vivo is proliferation of pre-existing infected T cells, rather than the *de novo* infection of new T cells. This also suggests that active production of infectious virions is not the major contributor to HTLV-1 pathogenesis (Gillet et al., 2011; Wattel et al., 1995). Finally, a number of groups have isolated and characterized CD4 T cell lines derived from HTLV-1 infected patients that express either no or only very low levels of HTLV-1 RNA and proteins constitutively (Lin et al., 1998; Richardson et al., 1997), suggesting that such latently infected cells also exist in vivo. Development of disease however appears to require Tax expression (Nomura et al., 2004; Peloponese et al., 2007; Yamagishi and Watanabe, 2012), at least in the early stages. Thus, activation of HTLV-1 expression from latently-infected cells and escape from the CTL response would likely be essential steps in HTLV-1 disease pathogenesis. A key question is how does HTLV-1 expression become activated in latent/low level-expressing, infected T cells?

The regulation of HTLV-1 gene expression is complex and involves both cellular and virally-encoded regulators. In particular, the viral Tax gene product, required for HTLV-1 transformation of CD4 T cells (reviewed in Matsuoka and Jeang (2011)), is a transcriptional activator of the HTLV-1 long terminal repeat (LTR), as well as of thousands of cellular genes (Ng et al., 2001). Tax has been extensively studied and transactivates the LTR by binding to cyclic AMP response element binding protein (CREB/ATF) family members (reviewed in Kashanchi and Brady (2005)), recruiting transcriptional coactivators including CBP and p300, and clearing occluding nucleosomes from the HTLV LTR (Lemasson et al., 2006; Nyborg et al., 2010). HTLV-1 gene expression is also controlled at the RNA level through the effects of the HTLV-1 Rex protein, which promotes nuclear export of unspliced and singly spliced RNAs (Baydoun et al., 2008; Nakano and Watanabe, 2012), and through the effects of p30 which inhibits nuclear export of *tax/rex* mRNAs (Anupam et al., 2013; Younis et al., 2006). The sum of these effects is early expression of the Tax and Rex proteins due to early export of *tax/rex* mRNAs, with later expression of structural and enzymatic proteins (Baydoun et al., 2008; Cavallari et al., 2013; Hidaka et al., 1988; Li et al., 2009). Important roles for the HTLV-1 anti-sense encoded gene, HBZ, have also been proposed (Barbeau and Mesnard, 2011; Matsuoka and Jeang, 2011; Mesnard et al., 2006). HBZ inhibits Tax-induced activation of the 5'LTR through inhibiting interactions with CREB proteins and CBP (Clerc et al., 2008; Gaudray et al., 2002; Lemasson et al., 2007), antagonizes the senescence-inducing properties of Tax (Zhi et al., 2011), which potentiates viral persistence (Arnold et al., 2006) and cellular proliferation (Arnold et al., 2008; Satou et al., 2006). Thus, the interplay of Tax, Rex, and HBZ is essential for transformation and disease progression (Matsuoka and Yasunaga, 2013), and plays a key role in regulation of HTLV-1 expression in some models of viral latency (Philip et al., 2014).

Several groups have studied the activation of expression of integrated HTLV-1 proviruses in latently infected CD4 T cells. These cell lines exhibit only low levels of HTLV-1 protein expression basally, but show marked activation of HTLV-1 protein expression upon treatment with various mimetics of different aspects of T cell activation. These include stimulation through the T cell receptor (TCR) by anti-CD3 antibody (Lin et al., 2005; Swaims et al., 2010), anti-CD2 antibody (Guyot et al., 1997), lectin [phytohemagglutinin-P (PHA), a surrogate for TCR cross-linking], phorbol ester [phorbol 12-myristate 13-acetate (PMA) which activates PKC pathways downstream of TCR activation (Lin et al., 1998)], and anti-CD28 antibody plus prostaglandins (Dumais et al., 2003). Cellular stresses (including arsenic, oxidative stress, heavy metal) (Andrews et al., 1995) and direct chromatin remodeling agents such as histone deacetylase inhibitors (Lemasson et al., 2004; Lin et al., 1998; Villanueva et al., 2006) also activate the HTLV-1 LTR.

In our current study, we observed a rapid but transient induction of *tax/rex* mRNA and protein following activation of infected T cells. We further examined the mechanism of HTLV-1 RNA induction and identified a marked enhancement of *tax/rex* mRNA stability following PMA treatment of chronically-infected, low-level expressing CD4 T cells, but not in infected cells with high basal levels of HTLV-1 expression. Thus, we propose that PMA activation may enhance the stability of *tax/rex* mRNA in latent/low-level expressing HTLV-1 infected cells, which in turn results in increased transcription from the HTLV-1 LTR, contributing to the exit of infected cells from latency and the induction of events ultimately contributing to HTLV-1 pathogenesis.

2. Materials and methods

2.1. Plasmids

Construction of pLTR-Luc (luciferase) was described previously (Lin et al., 1998). The human ASL promoter (−310 to +59) was amplified by polymerase chain reaction performed on genomic DNA purified from Jurkat T cells using Taq DNA polymerase (Qiagen,) and the following primer pair: cacctcgagcgggcctgatgtcatagcctctacc (forward) and gtcaagcttctccgcctggccgcacg (reverse). The amplified product was ligated into the *Xho*I and *Hin*dIII sites of pGL3-Basic (Promega) to create pASL-Luc. The *Nco*I/*Bam*HI fragment encoding the Tax gene, 3'LTR and SV40 polyadenylation site from pHTLV-tat1 (Nerenberg et al., 1987) was cloned in pLTR-Luc replacing the Luc gene, to create pLTR-Tax. The Tax gene and 3'UTR were deleted from pLTR-Tax by *Nco*I/*Bgl*II digestion, followed by treatment wit*h Kl* enow enzyme and religation to create pLTR-deltaTax (pLTR-DTax). Restriction enzymes and DNA modifying enzymes were obtained from New England Biolabs.

2.2. Cell lines and media

Except as noted below, cell lines were grown in RPMI 1640 (Life Technologies) supplemented with 10% fetal bovine serum (Gemini Bio-Products), 1% Penicillin/ Streptomycin (Life Technologies), 1% L-Glutamine (Life Technologies), and 10 mM HEPES (Life Technologies). JPX-9 cells (Nagata et al., 1989) were supplemented with 0.6 mg/ml G418 (Life Technologies). When indicated, JPX-9 and Jurkat cells [JE6-1 clone, (Weiss et al., 1984)] were treated with 5 μg/ml PHA (Sigma-Aldrich) and/or 120 μM zinc chloride (Sigma-Aldrich) for 24 h. FS and SP cells (Rowe et al., 1995) were grown in media containing 20% FBS and 20 U/ml IL-2. Transfections were performed using GenePORTER (Gene Therapy Systems) according to the manufacturer's protocol. Unless otherwise indicated, each reaction contained 400 ng luciferase reporter plasmid and 100 ng Tax (or deltaTax) plasmid. Transfected cells were treated with PHA (5 μg/ml; Sigma-Aldrich) or PMA (50 ng/ml; Sigma-Aldrich) four hours after transfection. Cells were harvested 24 h after transfection, washed in DPBS, resuspended in lysis buffer and assayed for luciferase activity using the manufacturer's protocol (Promega). Protein concentration was determined by the BCA method (Pierce). Luciferase activity was normalized to the amount of recovered protein for each sample. FS, SP, MT2, MT4 and HUT102 cells were treated with PMA (50 ng/ml) or vorinostat (5 μM; Sigma-Aldrich) for the indicated times, spun, washed and resuspended in fresh media. Cells were harvested at the indicated time points.

For studies of the effects of downstream pathways of protein kinase C (PKC) signaling on HTLV-1 activation, FS cells were pre-treated with the NF-κB inhibitor, Bay 11-7082 (5 μM, Cayman Chemical) (Pierce et al., 1997), the NFAT inhibitor, Cyclosporin A (0.5 μM, Cayman Chemical) (McCaffrey et al., 1993), or the AP-1 inhibitor, SR 11302 (1 μM, Cayman Chemical) (Fanjul et al., 1994) for 1 h, followed by a 15 min PMA treatment. Cells were then washed, resuspended with fresh media and cultured for 4 h. RNA were prepared as described.