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A new sensitive and quantitative HTLV-I-mediated cell fusion assay in T cells

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Abstract

Similar to several other viruses, human T cell leukemia virus type I (HTLV-I) induces the formation of multinucleated giant cells (also known as syncytium) when amplified in tissue culture. These syncytia result from the fusion of infected cells with uninfected cells. Due to the intrinsic difficulty of infecting cells with cell-free HTLV-I virions, syncytium formation has become an important tool in the study of HTLV-I infection and transmission. Since most HTLV-I-based cell fusion assays rely on the use of non-T cells, the aim of this study was to optimize a new HTLV-I-induced cell fusion assay in which HTLV-I-infected T cell lines are co-cultured with T cells that have been transfected with an HTLV-I long terminal repeat (LTR) luciferase reporter construct. We demonstrate that co-culture of various HTLV-I-infected T cells with different transfected T cell lines resulted in induction of luciferase activity. Cell-to-cell contact and expression of the viral gp46 envelope protein was crucial for this induction while other cell surface proteins (including HSC70) did not have a significant effect. This quantitative assay was shown to be very sensitive. In this assay, the cell fusion-mediated activation of NF-κB and the HTLV-I LTR occurred through previously described Tax-dependent signaling pathways. This assay also showed that cell fusion could activate Tax-inducible cellular promoters. These results thus demonstrate that this new quantitative HTLV-I-dependent cell fusion assay is versatile, highly sensitive, and can provide an important tool to investigate cellular promoter activation and intrinsic signaling cascades that modulate cellular gene expression.

Keywords: HTLV-I; Cell fusion; Tax; Luciferase

Introduction

HTLV-I is the etiological agent of adult T cell leukemia/lymphoma (ATLL) and tropical spastic paraparesis (TSP) also known as HTLV-I-associated myelopathy (HAM) (Gessain et al., 1985; Osame et al., 1986; Poiesz et al., 1980). Other documented complications associated with this virus include uveitis, cutaneous lymphoma, arthritis, and Sjögren's syndrome (Manns et al., 1999; Masuko-Hongo et al., 2003; Watanabe, 1997). Although this virus is prevalent in certain areas of Japan, very few infected individuals (from 2% to 5%) develop symptoms associated with HTLV-I infection. Furthermore, most of these associated diseases take decades to develop following infection. Because of the long clinical latency associated with HTLV-I infection, there is a lack of information regarding the onset of these illnesses as well as the mode of virus replication. However, it is known that in vivo HTLV-I proviral DNA is predominately found in CD4+ and CD8+ T cells (Richardson et al., 1990).

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In cell culture, it appears that HTLV-I tropism extends to other cell types or species of origin (Okuma et al., 1999; Trejo and Ratner, 2000). Recent identification of the putative HTLV-I receptor, the GLUT-1 glucose transporter, may explain the ubiquitous nature of HTLV-I tropism in vitro (Manel et al., 2003a).

It is well established that purified HTLV-I virions infect susceptible cells very inefficiently (Derse et al., 2001; Fan et al., 1992) and that transmission in cell culture is greatly improved when cell-to-cell contact is allowed. HTLV-I-infected cells undergo cell-to-cell fusion events (also termed syncytia) in cell culture. Although their existence in vivo is debatable, the importance of syncytia in HTLV-I infection and the role of cell surface proteins in viral entry have been well documented. As in viral infection, the viral gp46 glycoprotein mediates cell fusion by serving as the receptor interacting molecule and allows subsequent exposure of the fusion peptide to the viral gp21 transmembrane protein (Le Blanc et al., 2001; Sommerfelt, 1999). In addition to interaction of the cell receptor with gp46, other cellular proteins are likely to contribute to syncytium formation. It has been suggested that the heat shock cognate protein 70 (HSC70) might be an important player in HTLV-I-induced syncytium formation (Sagara et al., 1998). Similar to anti-gp46-blocking antibodies or sera from HTLV-I-seropositive patients, antibodies against certain other molecules also inhibit HTLV-I-induced syncytium formation (Daenke et al., 1999; Nagy et al., 1983; Okuma et al., 1999). For example, the use of antibodies against vascular cell adhesion molecule-1 (VCAM-1) and major histocompatibility complex class II (MHC-II) suggests that those molecules play a role in cell-to-cell fusion (Hildreth, 1998; Hildreth et al., 1997).

The viral Tax protein is also likely to affect syncytium formation. Tax has been clearly implicated in HTLV-I-induced cell transformation (Nerenberg et al., 1987), as well as playing a crucial role in HTLV-I replication. Indeed, Tax enhances the binding of the CREB transcription factor to elements in the HTLV-I LTR termed Tax responsive element 1 (TRE1) (Franklin et al., 1993; Zhao and Giam, 1992) and also recruits CREB-binding protein (CBP) to these complexes (Kwok et al., 1996). Because of its ability to activate CREB as well as the transcription factors NF-κB and SRF (Armstrong et al., 1993; Munoz and Israel, 1995; Yoshida, 1994), Tax is an important modulator of cellular gene expression. Specifically, Tax modulates the expression of cell adhesion molecules (Owen et al., 1997; Valentin et al., 2001), which are important factors in HTLV-I-mediated syncytium formation.

The study of HTLV-I-mediated syncytium formation requires maintenance of typical cell adhesion molecule interactions. Since several syncytium formation assays rely on non-T cells, we sought to develop a new quantitative system to evaluate HTLV-I-dependent syncytium formation in T cell lines. Using an HTLV-I LTR-dependent luciferase gene reporter system transfected into Jurkat T cells, we have quantitated the extent of syncytium formation between HTLV-I-producing T cell lines and Jurkat T cells by measuring luciferase activity. Our results show that this assay is very sensitive and provides a useful tool to study Tax-dependent modulation of cellular gene expression. The results further suggest that HSC70 is not important in this syncytium formation assay.

Results

Induction of HTLV-I LTR activity by co-cultured MT2 cells

Several assays have been designed to evaluate HTLV-I infection or HTLV-I-mediated cell-to-cell fusion events (Daenke et al., 1999; Igakura et al., 2003; Okuma et al., 1999). With this in mind, we wanted to develop a sensitive and quantitative method to evaluate HTLV-I-dependent cell fusion events that would exclusively involve T-cell-derived lines. This is an important feature of our assay since T lymphocytes are considered to be the primary cell type to harbor HTLV-I proviral DNA in vivo (Richardson et al., 1990). We reasoned that cell fusion in T lymphocytes might be easily quantitated using a construct containing the HTLV-I LTR regulating expression of the luciferase reporter gene (pHTLV-Luc). Hence, transfected cells that fuse with HTLV-I-infected cells should produce higher luciferase activity due to transactivation by the Tax protein provided by the infected cells.

To test whether co-culture could upregulate luciferase activity in T cells, we first incubated MT2 cells, which actively produce HTLV-I particles, with Jurkat cells that had been transfected with the pHTLV-Luc plasmid. Luciferase activity was measured at different time points following incubation. As presented in Fig. 1A, the addition of MT2 cells to pHTLV-Luc-transfected Jurkat cells resulted in a time-dependent induction of luciferase activity that reached a 48-h optimum of 114-fold and then diminished after 72 h of incubation. Induction of luciferase activity was detected as early as 8 h following co-culture, suggesting that the mechanism of induction involved cell-to-cell fusion processes as opposed to infection, which would require a longer time. To confirm these results, Jurkat cells transfected with pHTLV-Luc were cultivated for 24 h in the presence of HTLV-I-infected T cell lines producing viral particles (MT2) or not (C8166-45 and MT4). As shown above, the MT2 cell line induced luciferase activity, while the other non-producing cell lines had no effect on luciferase activity (Fig. 1B). To further confirm the ability of HTLV-I-infected cells to induce luciferase expression in uninfected cells, two additional HTLV-I-infected cell lines (MJ and C91/PL) were co-cultured with Jurkat cells that had been transfected with pHTLV-Luc (Fig. 1C). Increased luciferase activity was again observed following co-culture with both cell types although at the level of luciferase activation was variable. Although co-culture with C91/PL cells resulted in the greatest induction of luciferase activity,