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Vpu-mediated tetherin antagonism of ongoing HIV-1 infection in CD4$^+$ T-cells is not directly related to the extent of tetherin cell surface downmodulation

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ABSTRACT

Tetherin is a host cell restriction factor that acts against HIV-1 and other enveloped viruses. The antiviral activity of tetherin is antagonized by the HIV-1 protein Vpu, that downregulates tetherin from the cell surface. Here, we report the specific detection of cell surface tetherin levels in primary activated CD4$^+$ T-cells and in CD4$^+$ T-cell lines. Differences were observed regarding tetherin cell surface expression, Vpu-mediated tetherin downmodulation and promotion of virus release. However, Vpu expression in all T-cell lines resulted in a 2-fold increase in numbers of infected cells after three days. This implies a Vpu-mediated effect in ongoing infection and possibly in cell-to-cell viral spread that is independent of the extent of Vpu-mediated tetherin cell surface downmodulation. Endogenous cell surface tetherin levels in T-cell lines were also down-modulated following infection with Vpu-deleted virus, suggesting an additional Vpu-independent mechanism of tetherin cell surface downmodulation following HIV-1 infection in T-cell lines.

Introduction

Tetherin (BST-2/CD317/HM1.24) is a host cell restriction factor that contributes to cellular defense against infection by HIV-1 and other enveloped viruses; tetherin-mediated restriction is interferon responsive (Jouvenet et al., 2009; Neil et al., 2008; Van Damme et al., 2008). In HIV-1 infections, the viral protein Vpu antagonizes tetherin-mediated restriction and promotes virus release (Neil et al., 2008; Van Damme et al., 2008). The antiviral action of tetherin is due to its presence in the membrane of budding viral particles, tethering nascent viral particles to the cell surface and to each other (Kupzig et al., 2003; Neil et al., 2008; Perez-Caballero et al., 2009; Van Damme et al., 2008). At the cell surface, tetherin localizes to lipid rafts (Goffinet et al., 2009; Kupzig et al., 2003; Rollason et al., 2009, 2007), which, during the HIV-1 life cycle are the focus of viral assembly, budding, as well as entry; lipid rafts are involved in both cell-free virus spread and direct cell-to-cell spread (reviewed in (Waheed and Freed, 2009)). Direct cell-to-cell spread is reported to increase the efficiency of HIV-1 transmission by 100–18,000 times compared to cell-free spread and is considered to be the predominant mode of HIV-1 spread in T-cell lines and in secondary lymphoid tissue (Chen et al., 2007; Dimitrov et al., 1993; Gummuluru et al., 2000; Hübner et al., 2009; Sourisseau et al., 2007) (reviewed in (Mothes et al., 2010; Sattentau, 2008)). In addition to restricting virus release and subsequently cell-free viral spread, we and others have shown that tetherin also inhibits direct cell-to-cell transmission in T-cells (Casartelli et al., 2010; Kuhl et al., 2010b). Others have reported that HIV-1 might overcome tetherin-mediated restriction of direct cell-to-cell viral spread (Jolly et al., 2010).

The capacity of tetherin to restrict virus release is commonly attributed to its cell surface expression. Vpu activity in counteracting tetherin-mediated restriction is believed to result from Vpu-mediated tetherin cell surface down-regulation, which either results from tetherin degradation or from its sequestration in intracellular compartments (Dubé et al., 2010; Dube et al., 2009; Goffinet et al., 2009; Iwabu et al., 2009; Mangeat et al., 2009; Perez-Caballero et al., 2009; Van Damme et al., 2008). Most of these data were obtained using the HeLa epithelial cell line, which expresses high endogenous levels of tetherin or with the 293T human embryonic kidney cell line, which naturally lacks tetherin expression and must be transfected with tetherin-expressing plasmids.

The specific detection of tetherin expression at the cell surface has been reported for only a few cell lines (HeLa, MT-4, COS-7) and for primary B-cells, plasmacytoid dendritic cells (pDCs) and monocyte derived macrophages (MDMs) (Blasius et al., 2006; Mitchell et al.,

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2009; Miyagi et al., 2009; Rong et al., 2009; Sato et al., 2009; Van Damme et al., 2008; Vidal-Laliena et al., 2005). Interferon-α (IFNα) increased cell surface expression in these cells and also induced detectable cell surface expression in 293T cells (Van Damme et al., 2008). In peripheral blood mononuclear cells (PBMCs), total cellular tetherin expression had previously only been shown after IFNα-treatment by Western blot of cell lysates (Miyagi et al., 2009).

It was recently reported that endogenous tetherin is differentially modified at the post-translational level compared to tetherin that is derived from an exogenous source (Andrew et al., 2010). Cell-line specific differences have been reported for expression patterns of other host cell restriction factors, such as APOBEC3G, which also confers resistance to HIV-1 infections and which is antagonized by the viral accessory protein Vif (reviewed in (Henriet et al., 2009; Niewiadomska and Yu, 2009)). APOBEC3G is expressed and restricts viral replication in CEM-CCRF cells but not in a derivative cell line CEM-SS (Foley et al., 1965; Sheehy et al., 2002).

Few studies have investigated the relationship between cell surface tetherin expression and virus release in infected T-cell lines (Miyagi et al., 2009; Rong et al., 2009; Sato et al., 2009). While virus release might be attributed to expression levels of cell surface tetherin in MT-4 (Harada et al., 1985; Sato et al., 2009) and stably transduced Sup-T1 cells (Rong et al., 2009), other work that used the H9 T-cell line and the CEMx174 T/B-cell fusion cell line reported tetherin-mediated restriction that was independent of tetherin cell surface levels, suggesting the possibility of cell-type specific differences in the effect of tetherin on virus release (Miyagi et al., 2009). To address this, we determined tetherin cell surface expression in relation to virus release and infection rates. Here, we report specific detection of cell surface tetherin expression in primary activated $CD4^+$ T-cells and in multiple T-cell lines. Strong differences in regard to tetherin cell surface expression, Vpu-mediated tetherin downmodulation, and promotion of virus release were observed among them. We show that the influence of Vpu on multiple-round infections was equivalent in all T-cell lines, and that twice as many cells were infected at 72 h post infection (p.i.) in the case of *vpu*-containing compared to Δ*vpu* infections. This implies a tetherin-mediated effect on cell-to-cell spread that is not directly related to its cell surface expression. In addition, we report a Vpu-independent downregulation of endogenous tetherin following infection of $CD4^+$ T-cell lines.

Results

Variation of tetherin cell surface expression in T-cell lines

We first assessed the cell surface expression of endogenous tetherin by flow cytometry in CEM-CCRF, CEM-SS, and H9 cells, in addition to Sup-T1 cells stably transduced with human tetherin (Kuhl et al., 2010b; Rong et al., 2009). We were able to specifically detect and assess cell surface expression of tetherin in all of these cell lines (Fig. 1A). Cell surface expression of tetherin varied between the cell lines; relative mean expression levels were 9.1 in H9 cells, 23.4 in CEM-CCRF cells and 44.5 in CEM-SS cells. In the case of the tetherin-inducible Sup-T1 cell line, we employed a doxycycline titration method to specifically induce cell surface tetherin levels that resemble levels detected on T-cell lines (Figs. 1A and B). Induction with 5 ng/ml doxycycline resulted in a relative mean cell surface expression of tetherin of 8.3, which was similar to that obtained in H9 cells.

We also assessed cell size and cell granularity/complexity by flow cytometric analysis of forward scatter (FSC) and side scatter (SSC), respectively. Side scatter patterns differed between cell types due to differences in cell granule content which in combination with FSC is a commonly used characteristic for identification of cell populations. All cell lines tested here were of similar size (FSC) and granularity/complexity (Figs. 1C and D).

Cell line specific differences of vpu-mediated tetherin downmodulation

Next, we assessed the capacity of Vpu to downregulate cell surface expression of tetherin following HIV-1 infection in the various cell lines. Cells were infected to a level of ~10%, as assessed by flow cytometric detection of eGFP expression at 48 h p.i., with single-round infections, *i.e. env*-deleted, *wt* (*vpu*-positive), or Δ*vpu* (*vpu*-deleted) virus, pseudotyped for entry with the Vesicular stomatitis virus protein G (VSV-G) envelope. Tetherin cell surface expression in infected (eGFP positive) and uninfected (eGFP negative) cells was determined by flow cytometry. In the stably transduced Sup-T1 cell line infected with *wt* virus, tetherin cell surface levels were significantly downregulated compared to uninfected controls (~40%). Uninfected H9 cells showed similar tetherin cell surface expression as did uninfected Sup-T1 cells induced with 5 ng/ml doxycycline (Figs. 2A and B). However, infection of H9 cells with *wt* virus resulted in only a modest tetherin down-regulation (~27%) compared to uninfected cells (Fig. 2A). CEM-SS cells exhibited significantly higher tetherin cell surface levels than did the parental CEM-CCRF cell line (Figs. 2C and D). In both these cell lines, cell surface tetherin was downregulated in *wt* infection but to different extents (CEM-SS: ~78%; CEM-CCRF: ~45%).

Vpu-independent tetherin modulation

In CEM-SS cells, cell surface tetherin was also downregulated after infection by Δ*vpu* virus, compared to uninfected controls (~47%) (Fig. 2D); similar trends were observed with CEM-CCRF (~33%) and H9 cells (~13%) (Figs. 2A and C). This effect was not observed in the transduced Sup-T1 cell line (induced with 5 ng/ml doxycycline); such cells, when infected with Δ*vpu* virus, showed a slight, but statistically insignificant upregulation of tetherin (~15%) (Fig. 2B).

Cell line specific effect of Vpu-mediated tetherin modulation on virus release

To assess the effect of Vpu on virus release we infected the cell lines with equal amounts of *wt* or Δ*vpu* virus, based on CA p24 levels, and measured virus release into the supernatant at 24, 48 and 72 h p.i. using a quantitative reverse transcription-based assay (Fig. 3). Virus release at baseline (24 h p.i.) was similar in all infections. Starting at 48 h p.i. Vpu mediated increased levels of virus release in all cell lines, though the effect differed among them. At 72 h p.i., the extent of the Vpu effect on virus release (comparing *wt* and Δ*vpu*) ranged from ~60% in H9 cells to ~550% in CEM-SS cells (CEM-CCRF: ~170%; induced Sup-T1: ~300%) (Fig. 3). In order to compare the direct effect of Vpu on tetherin cell surface downmodulation and virus release, we normalized the Vpu-mediated increase of virus release to the extent of Vpu-mediated tetherin cell surface downregulation (Table 1). A greater ratio implies a stronger correlation of virus release and changes in tetherin cell surface expression. The similarity of the ratios between CEM-CCRF cells (10.2) and derivative CEM-SS cells (10.5) shows that tetherin-mediated restriction of virus release was strongly affected by tetherin cell surface expression levels in both instances. H9 cells showed a low ratio (3.5), indicating a lower correlation between tetherin-mediated restriction of virus release and cell surface expression, while inducible Sup-T1 cells showed an intermediate level of correlation (6.1).

Similar influence of Vpu in ongoing infection

Using flow cytometry detection of virus-derived eGFP expression at 72 h p.i., we assessed cell line susceptibility to *wt* virus. Infections with equal amounts of *wt* virus, based on CA p24 levels, resulted in infection rates ranging from ~1% in H9 cells to ~40% in inducible Sup-T1 cells (CEM-SS: ~3%; CEM-CCRF: ~20%) (Fig. 4). The presence of Vpu (comparing *wt* and Δ*vpu* virus) increased infection rates at 72 h p.i. to similar levels in all cell lines, ranging from a 107% increase in CEM-CCRF