Virology 498 (2016) 192–200

Contents lists available at ScienceDirect

Virology

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

HIV-1 tat protein recruits CIS to the cytoplasmic tail of CD127 to induce receptor ubiquitination and proteasomal degradation

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ARTICLE INFO

Article history:
Received 2 June 2016
Returned to author for revisions
25 August 2016
Accepted 26 August 2016

Keywords:
AIDS
HIV-1
Tat
IL-7
CD8 T cell
CIS
SOCS

ABSTRACT

HIV-1 Tat protein down regulates expression of the IL-7 receptor alpha-chain (CD127) from the surface of CD8 T cells resulting in impaired T cell proliferation and cytolytic capacity. We have previously shown that soluble Tat protein is taken up by CD8 T cells and interacts with the cytoplasmic tail of CD127 to induce receptor degradation. The N-terminal domain of Tat interacts with CD127 while the basic domain directs CD127 to the proteasome. We have also shown that upon IL-7 binding to its receptor, CD127 is phosphorylated resulting in CIS-mediated proteasomal degradation. Here, we show that Tat mimics this process by recruiting CIS to CD127 in the absence of IL-7 and receptor phosphorylation, leading to CD127 ubiquitination and degradation. Tat therefore acts as an adapter to induce cellular responses under conditions where they may not otherwise occur. Thusly, Tat reduces IL-7 signaling and impairs CD8 T cell survival and function.

1. Introduction

While CD8 T cells play a major role in the initial containment of HIV viremia (Borrow et al., 1994; Goonetilleke et al., 2009; Steeper et al., 1988), the progressive loss of CD8 T cell function and impairment of cell mediated immunity characterize HIV disease progression (Gerstoft et al., 1985; Sharma and Gupta, 1985). How HIV limits CD8 T cell activity is likely multi-factorial but one possible mechanism is through altered IL-7 signaling. IL-7 is a key cytokine in T cell biology and we have shown previously that expression of the IL-7 receptor alpha-chain (CD127) is down regulated on the surface of CD8 T cells isolated from HIV infected individuals (MacPherson et al., 2001). This finding is significant as IL-7 promotes CD8 T cell effector proliferation and function by up regulating the expression of telomerase (Soares et al., 1998), the cytolytic molecule perforin (Smyth et al., 1991), the second-signal molecule CD69, and the high affinity component of the IL-2 receptor (CD25) (Park et al., 2007; Swainson et al., 2006), as well as promoting transition through the cell cycle (Liu et al., 2006) and inducing the translocation of glucose transporter (GLUT)-1 to the cell membrane (Swainson et al., 2007). Since IL-7 signaling promotes effector proliferation and function, many of the CD8 T cell deficiencies seen in HIV infection could be explained at least in part by reduced IL-7 signaling.

HIV encodes for the 14 kDa transactivator of transcription (Tat) protein named for its canonical role in enhancing transcription of the HIV genome from the LTR promoter region. Interestingly, Tat is secreted from HIV infected CD4 cells (Rayne et al., 2010a, 2010b) and can be readily detected in culture supernatants in vitro as well as in the tissues and blood plasma of HIV infected individuals (Poggi et al., 2004; Westendorp et al., 1995; Xiao et al., 2000). The half-life of Tat in the blood plasma is very short as it is rapidly taken up by uninfected cells (Fittipaldi and Giacca, 2005; Helland et al., 1991) where it elicits a variety of biological effects (Debaisieux et al., 2012; Huigen et al., 2004). We have shown that Tat down regulates CD127 from the surface of primary CD8 T cells isolated from healthy HIV-negative individuals (Faller et al., 2006) and maintains suppression of CD127 on CD8 T cells isolated from HIV positive individuals (Faller et al., 2014). Tat first binds heparan sulfate proteoglycans on the surface of T cells and is then internalized through clathrin coated pits (Vendeville et al., 2004). As Tat travels through the endocytotic pathway it is able to gain access to the cytosol through a process dependent on the usual acidification of late endosomes (Vendeville et al., 2004; Yezid et al., 2009). Once in the cytosol, Tat interacts with the cytoplasmic

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http://dx.doi.org/10.1016/j.virol.2016.08.024
0042-6822/

tail of CD127 located in early recycling endosomes and directs the receptor to the proteasome for degradation (Faller et al., 2010). Tat's effect on CD127 is specific. We have shown Tat does not affect the expression of other cell surface proteins including CD25, CD45, CD56 or CD132 (Faller et al., 2010). We have also shown Tat does not affect *de novo* CD127 synthesis or the trafficking of the receptor from the endoplasmic reticulum to the cell membrane. Further, the effect of Tat on CD127 expression is not mediated through apoptosis or cellular activation (Faller et al., 2010). Importantly, Tat-mediated CD127 down regulation results in impaired CD8 T cell proliferation and perforin production in response to IL-7 stimulation (Faller et al., 2006).

We have recently shown the N-terminal and basic regions of Tat are required to down regulate CD127 from the surface of CD8 T cells (Sugden and MacPherson, 2015). Specifically, Tat protein lacking the N-terminal region is neither able to interact with nor down regulate CD127 from the cell surface. In contrast, Tat mutants lacking the basic region (ΔBasic Tat) are able to interact with the receptor but are unable to remove CD127 from the cell membrane. Taken together, these data suggest that Tat interacts with CD127 via its N-terminal region and recruits a yet undetermined cellular factor to the receptor via its basic region to accelerate the internalization and degradation of CD127 (Sugden and MacPherson, 2015).

We have also recently elucidated the pathway by which IL-7 regulates cell surface expression of CD127 on CD8 T cells (Faller et al., 2015; Ghazawi et al., 2013). IL-7 binding to CD127 triggers receptor internalization through clathrin coated pits resulting in receptor accumulation in early endosomes. IL-7 binding also activates JAK3 which phosphorylates tyrosine 449 (Y449) in the cytoplasmic tail of CD127 as well as STAT5 which in turn translocates to the cell nucleus where it up regulates expression SOCS proteins SOCS2 and *Cytokine-inducible* SH2 containing (CIS) protein. CIS and SOCS2 then associate with CD127 phosphorylated at Y449 via their SH2 domains and traffic with the receptor to the proteasome. This CIS-dependent degradation of CD127 in response to IL-7 is dependent on cellular E3 ubiquitin ligase strongly implicating ubiquitination in this process.

We show here that soluble Tat protein taken up by CD8 T cells interacts with CD127 and recruits CIS protein to the receptor resulting in ubiquitination of CD127. Consistent with our previous findings, the association of CIS with Tat is dependent on Tat's basic domain. We propose a model whereby Tat acts as an adapter protein interacting with CD127 via its N-terminal region and recruits CIS via its basic region to the receptor in the absence of IL-7 and CD127 phosphorylation. This recruitment of CIS results in CD127 ubiquitination and targets the receptor to the proteasome for degradation.

2. Results

2.1. Tat does not induce CD127 phosphorylation at Y449

We have shown previously that Tat interacts with CD127 to down regulate surface expression and direct the receptor to the proteasome for degradation (Faller et al., 2006, 2010). By comparison, IL-7 binding to its receptor results in the phosphorylation of CD127 at Y449 and we have shown phosphorylation at this tyrosine residue targets the receptor to the proteasome (Ghazawi et al., 2013). In view of this and since Tat is known to interact with several cellular kinases (Cai et al., 2000; Gee et al., 2007; Leghmari et al., 2008), we questioned whether Tat directs CD127 degradation by inducing phosphorylation of the receptor in a manner similar to IL-7. To investigate this possibility, CD8 T cells were incubated with purified Tat protein for 12 h and then analyzed by western blot for CD127 phosphorylation at Y449. The 12 h time point was chosen as we have previously shown this is sufficient time for Tat to enter the cell and interact with CD127 but is prior to maximal degradation of the receptor (Faller et al., 2010). In parallel, CD8 T cells were treated with IL-7 for ten minutes as a positive control. As shown in Fig. 1, while IL-7 as expected induced phosphorylation of CD127 at Y449, Tat protein did not. These data indicate that Tat is not targeting CD127 for proteasomal degradation by inducing phosphorylation of Y449 in a manner similar to IL-7.

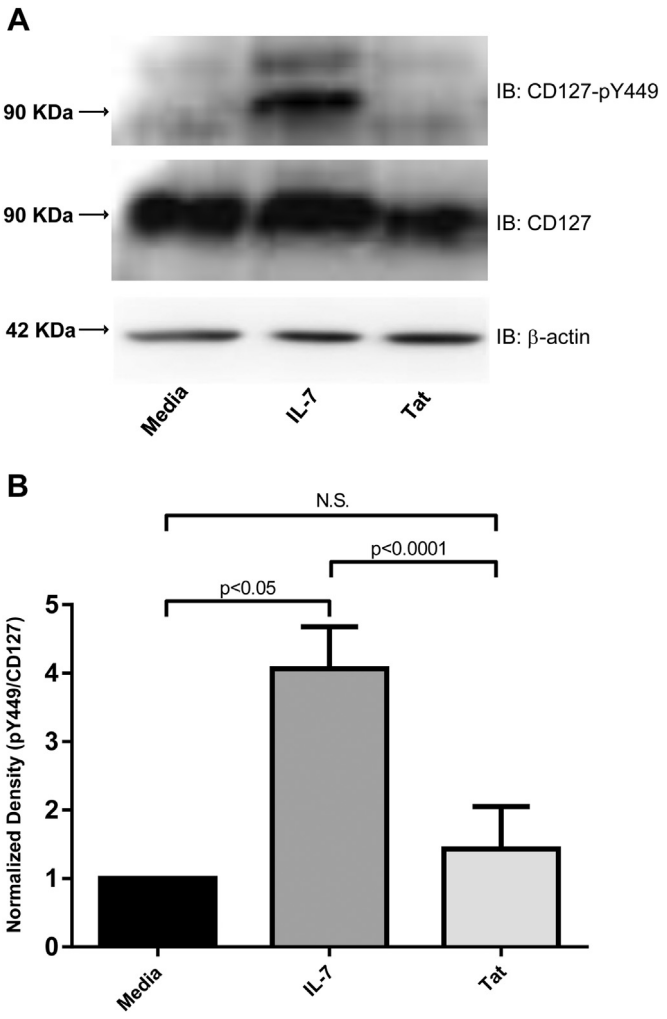

Fig. 1. Tat does not induce phosphorylation of CD127 at Y449. Primary human CD8 T cells were treated with either Tat (10 μg/mL) for 12 h, IL-7 (10 ng/mL) for 10 min, or left in medium alone. Cells were then lysed and analyzed by western blot for CD127 or CD127 phosphorylated at Y449. Blots were also probed for β-actin as a loading control. (A) Representative blots. (B) Bands were quantified via densitometry. Relative density of CD127 pY449 normalized to whole CD127 is shown. Error bars represent standard error of the mean (±). Statistical analysis was performed using a paired two-tailed student *t*-test (n=3).

2.2. Tat-mediated CD127 down regulation requires ubiquitin ligase machinery

We have recently shown that following IL-7 stimulation of CD8 T cells, CIS and SOCS2 interact with CD127 and recruit an E3 ubiquitin ligase to the receptor complex which in turn targets the complex to the proteasome (Ghazawi et al., 2016). Interestingly, Tat has been shown to interact with E3 ubiquitin ligases (Col et al., 2005; Kalantari et al., 2008) and itself is ubiquitinated (Bres et al., 2003; Hetzer et al., 2005). To investigate the potential role of