Virology 417 (2011) 50–56

Contents lists available at ScienceDirect

Virology

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

Influenza virus is not restricted by tetherin whereas influenza VLP production is restricted by tetherin

Rie Watanabe [1], George P. Leser, Robert A. Lamb *

Howard Hughes Medical Institute, Dept. of Molecular Biosciences, Northwestern University, Evanston, IL 60201-2138, USA

ARTICLE INFO

Article history:
Received 4 April 2011
Accepted 10 May 2011
Available online 28 May 2011

Keywords:
Influenza virus
Virus-like particles
Tetherin
BST2
CD317
Virus restriction
Restriction factors

ABSTRACT

Tetherin (ST2/CD317) is a cellular protein that restricts the release from cells of some enveloped viruses including HIV-1. To examine if influenza virus is affected by tetherin, MDCK cells constitutively expressing human tetherin and control MDCK cells were infected with influenza virus. No difference was observed in infectious titers, at 24 h or 48 h post-infection. In contrast, tetherin expression inhibited influenza virus-like particle (VLP) release into the media. Expression of the HIV protein Vpu overcame the tetherin block of influenza virus VLPs. A human tetherin mutant that lacks a C-terminal GPI anchor attachment signal (tetherin-ΔGPI) was constructed to test if this mutant could be incorporated into the released virus or VLP particles. Whereas tetherin-ΔGPI was incorporated into influenza VLPs it was not incorporated into influenza virions. Taken together these data suggest that influenza virions may contain a tetherin antagonist.

Introduction

To combat viral infections cells have developed a variety of strategies to restrict virus infections at various points in their life cycles. Tetherin (also known as CD317/BST-2/HN1.24) is an interferon-inducible integral membrane protein that contributes to the establishment of the anti-viral state; however, there is a basal constitutive level of expression in many cell types (reviewed in Evans et al., 2010).

Tetherin is a type II integral membrane protein with a cytoplasmic N-terminus and an extracellularly localized C-terminus that is post-translationally modified by addition of a glycosylphosphatidylinositol (GPI) membrane anchor. Thus, the tetherin molecule is anchored in the membrane at both of its termini. Tetherin is expressed at the plasma membrane and is localized to lipid rafts (Kupzig et al., 2003). Tetherin is a homodimer that is disulfide-linked through three extracellular cysteine residues. The ectodomain is also glycosylated by two N-linked carbohydrate chains that are heterogeneously modified (possibly by polylactosaminoglycan) that cause tetherin to migrate on SDS-PAGE as a smear of 28–45 KDa (Perez-Caballero et al., 2009).

The first enveloped virus shown to be restricted in its release from infected cells by tetherin was human immunodeficiency virus (HIV-1) (Neil et al., 2008; Van Damme et al., 2008). More recently tetherin has been shown to have a broad activity against diverse families of enveloped viruses including human immunodeficiency virus 2 (HIV-2), simian immunodeficiency virus (SIV), Ebola virus and Marburg virus, Lassa fever virus, vesicular stomatitis virus and Kaposi's sarcoma herpes virus (KSVH) (Jouvenet et al., 2009; Kaletsky et al., 2009; Radoshitzky et al., 2010; Sakuma et al., 2009; Weidner et al., 2010). Many viruses can overcome restriction of budding by tetherin using diverse viral proteins: Vpu for HIV-1; Env for HIV-2; Env/Nef interplay for SIV, GP for Ebola virus and protein K5 of KSHV (Gupta et al., 2009; Jia et al., 2009; Kaletsky et al., 2009; Le Tortorec and Neil, 2009; Mansouri et al., 2009; Neil et al., 2008; Van Damme et al., 2008). Vpu is thought to antagonize tetherin by removing it from the sites of virus assembly through internalization and proteasomal degradation (reviewed in Evans et al., 2010).

Many of the studies performed to analyze the role of tetherin in restricting the release of an enveloped virus have been performed using virus-like particles (VLPs). Recently it has been observed that whereas the release of Ebola virus VLPs are restricted by tetherin, infectious Ebola virus is not restricted by tetherin (Radoshitzky et al., 2010). This suggests that Ebola virions contain a tetherin antagonist probably excluding tetherin from the virions.

Tetherin is a lipid raft-associated apically-expressed membrane protein (Kupzig et al., 2003) and as influenza virus utilizes lipid rafts as a budding platform (Takeda et al., 2003) it was of interest to examine the effect of tetherin on influenza virus budding and on the budding of influenza VLPs. We found that whereas tetherin expression did not affect influenza virus budding, influenza VLP budding was restricted.

* Corresponding author at: Dept. Mol. BioSci, Northwestern University, 2205 Tech Drive, Evanston, IL 60208-3500, USA. Fax: +1 847 491 2467.
E-mail address: ralamb@northwestern.edu (R.A. Lamb).
[1] Present address: Department of Veterinary Medicine, Tokyo University of Agriculture and Technology, Tokyo, Japan.

0042-6822/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2011.05.006

Results

Influenza virus growth is not restricted by expression of tetherin

An MDCK cell line that constitutively expresses a N-terminally HA-tagged tetherin protein was generated. On SDS-PAGE, tetherin migrated heterogeneously (Fig. 1a), due to carbohydrate modification (Perez-Caballero et al., 2009), which we speculate is due to addition of polylactosaminoglycan. MDCK and MDCK-tetherin cells were infected with influenza virus A/Udorn/72 and A/WSN/33 at a multiplicity of infection of 1 plaque forming units (PFU)/cell and at 24 h and 48 h post-infection (p.i.) the infectivity of the released virus was determined. The virus titers were found to be very similar whether the virus was grown in MDCK or MDCK-tetherin cells (Fig. 1b). Analysis of the accumulation of virus-specific polypeptides in infected cells and in released virions at 24 h p.i. showed that they were comparable when influenza A/Udorn/72 virus was used to infect MDCK or MDCK-tetherin cells (Fig. 1c).

To compare influenza virus to another virus, vesicular stomatitis disease virus (VSV), that had been shown to be restricted by tetherin expression (Weidner et al., 2010) we used a trans-complementation strategy (Pawliczek and Crump, 2009). This was done to circumvent the viral titer derived from infected cells that were not transfected with DNA, due to the difficulty of being unable to transfect all the cells in a culture but being able to infect all the cells with virus. We used two defective viruses, influenza A/Udorn/M2S71 (Chen et al., 2008) and VSV/ΔG/GFP (Takada et al., 1997), a VSV that expresses green fluorescent protein in place of the G gene. Influenza A/Udorn/M2S71 was complemented by expression of M2 protein and VSV/ΔG/GFP was complemented by expression of G protein. 293 T cells were transfected with cDNA expressing tetherin and M2 or tetherin and G and 24 h later cells were infected with influenza A/Udorn/M2S71 or VSV/ΔG/GFP, respectively. For influenza virus, titers were measured by plaque assay on M2CK cells (Chen et al., 2007) and for VSV titers were determined by flow cytometry (Watanabe and Lamb, 2010). It was found that tetherin expression reduced VSV yield by 6-fold

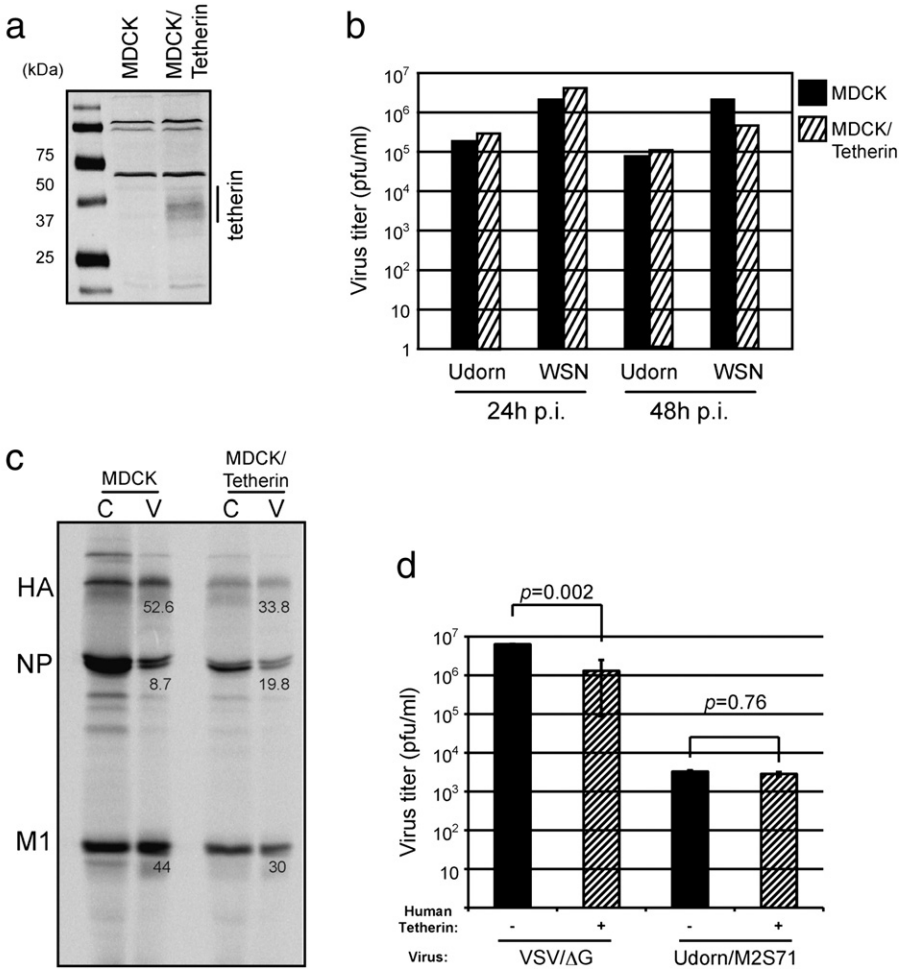

Fig. 1. Human tetherin expressed in MDCK cells does not restrict influenza virus budding. (a) Constitutive expression of HA-tagged Hu tetherin in MDCK cells. Cells were lysed in SDS-lysis buffer and polypeptides separated by SDS-PAGE followed by immunoblotting with anti-HA Ab to detect the HA-tagged tetherin. A bar indicates the heterogeneously migrating glycosylated tetherin species. (b) Infectious titer at 24 and 48 h p.i. of influenza virus (A/Udorn/72 and A/WSN/33) grown in MDCK or MDCK/tetherin cells. (c) Budding efficiency of wt Udorn from MDCK or MDCK/tetherin cells at 24 h p.i.. Infected cells were labeled with ^{35}[S] Trans-label and influenza virus-specific polypeptides in both cells and virions were immunoprecipitated using goat anti-Ud. C = intracellular polypetides; V = polypeptides released into media as virus. Numbers below bands indicate quantification of the released viral polypeptide as a percentage of total cell + virus. (d) Effect of tetherin on influenza virus and VSV budding using a trans-complementation assay. 293 T cells were plated onto gelatin-coated 6-well plate at a density of 0.3×10^6/well the day before transfection. 293 T cells were transfected with 0.5 μg plasmid DNA pCAGGS-M2 or pCAGGS-VSV G and pCAGGS tetherin. After 24 h incubation, cells were infected with influenza virus A/Udorn/M2S71 or VSV/ΔG/GFP/VSV G at MOI = 3.3. Culture supernatant was harvested at 24 h (A/Udorn/M2S71) or 7 h (VSV/ΔG) p.i. and the infectious titer was determined as described below. The infectious titer of influenza virus A/Udorn/M2S71 was determined by plaque assay using M2CK cells as described previously (Chen et al., 2007). To determine the infectious unit of VSV/ΔG/GFP, BHK cells were infected with serially diluted virus. Cells were harvested at 24 h p.i. and GFP (+) cells were detected by flow cytometry (FACSCalliber Becton Dickinson, Franklin Lakes, NJ). VSV infectious units were calculated based on the percentage of GFP (+) cells in the population.