Virology 406 (2010) 312–321

Contents lists available at ScienceDirect

Virology

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

BST-2 mediated restriction of simian–human immunodeficiency virus

Autumn Ruiz [a], David Lau [c], Richard S. Mitchell [c], M. Sarah Hill [b], Kimberly Schmitt [a], John C. Guatelli [c,d], Edward B. Stephens [a,b,*]

[a] Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas 66160, USA
[b] Department of Microbiology, Molecular Genetics, and Immunology, University of Kansas Medical Center, Kansas City, Kansas 66160, USA
[c] Department of Medicine, University of California San Diego, California 92093, USA
[d] The Veterans Affairs San Diego Healthcare System, San Diego, CA 92161, USA

ARTICLE INFO

Article history:
Received 6 April 2010
Returned to author for revision 27 April 2010
Accepted 14 July 2010
Available online 13 August 2010

Keywords:
HIV-1
Vpu
BST-2
Simian–human immunodeficiency virus
SHIV
Pig-tailed macaque
Rhesus macaque
Virus release
Transmembrane domain

ABSTRACT

Pathogenic simian–human immunodeficiency viruses (SHIV) contain HIV-1 Vpu and SIV Nef, both shown to counteract BST-2 (HM1.24; CD317; tetherin) inhibition of virus release in a species-specific manner. We show that human and pig-tailed BST-2 (ptBST-2) restrict SHIV. We found that sequential "humanization" of the transmembrane domain (TMD) of the pig-tailed BST-2 (ptBST-2) protein resulted in a fluctuation in sensitivity to HIV-1 Vpu. Our results also show that the length of the TMD in human and ptBST-2 proteins is important for BST-2 restriction and susceptibility to Vpu. Taken together, our results emphasize the importance of tertiary structure in BST-2 antagonism and suggests that the HIV-1 Vpu transmembrane domain may have additional functions *in vivo* unrelated to BST-2 antagonism.

Introduction

Human bone marrow stromal cell antigen 2 (BST-2; HM1.24; CD317 or tetherin) was recently identified as a potent inhibitor of the release of multiple enveloped viruses including human immunodeficiency virus type I (HIV-1) and simian immunodeficiency virus (SIV) (Jouvenet et al., 2009; Kaletsky et al., 2009; Neil et al., 2008; Van Damme et al., 2008). While the exact cellular function of human BST-2 (hBST-2) in the human host is unclear, it has been shown to play a role in regulating the growth and development of B cells, to be involved in the organization of the subapical actin cytoskeleton in polarized epithelial cells (Rollason et al., 2009), and most recently to function as a ligand for ILT7, a receptor that inhibits IFN production from plasmacytoid dendritic cells (Cao et al., 2009). BST-2 is an interferon-induced, lipid raft-associated, type II integral membrane protein with an unusual topology that is similar in membrane orientation to a neuro-pathogenic form of the prion protein (PrP) (Kupzig et al., 2003). It contains a short cytoplasmic N-terminus followed by a transmembrane domain (TMD), a central extracellular domain predicted to form a coiled-coil structure, and a C-terminal cleavage site predicted to form a glycosyl-phosphatidylinositol (GPI) anchor (Ishikawa et al., 1995; Kupzig et al., 2003). This topology supports membrane-spanning models of restriction of virion release, in which the protein would form dimers providing a physical, protease-sensitive link between the cellular and viral membranes (Perez-Caballero et al., 2009). This model is supported by evidence that hBST-2 is incorporated into nascent virions and that the formation of cysteine-linked dimers is required for hBST-2 restriction of HIV-1 virion release (Ali et al., 2010; Andrew et al., 2009; Fitzpatrick et al., 2010; Habermann et al., 2010; Perez-Caballero et al., 2009).

Several studies have provided evidence that viruses including HIV-1 and SIV have evolved to acquire activity against the anti-viral properties of BST-2. The HIV-1 group M Vpu proteins counteract specifically the restriction of human, chimpanzee and gorilla BST-2 proteins, with the exception of Vpu from strains JR-CSF and YU-2, which also antagonized BST-2 proteins derived from Greater-spot nosed and African green monkeys (Jouvenet et al., 2009; Neil et al., 2008; Sauter et al., 2009; Van Damme et al., 2008). The Nef proteins isolated from SIV_{mac}, SIV_{cpz}, SIV_{gor}, and SIV_{agm} also antagonize the effects of certain non-human primate BST-2 proteins, including those isolated from rhesus (rhBST-2) and pig-tailed (ptBST-2) macaques (Jia et al., 2009; Sauter et al., 2009; Zhang et al., 2009). Similar to other known intrinsic viral restriction factors such as the APOBEC3 and TRIM families of proteins, the ability of specific viral

* Corresponding author. Department of Microbiology, Molecular Genetics, and Immunology University of Kansas Medical Center, Kansas City, Kansas 66160, USA.
E-mail address: estephen@kumc.edu (E.B. Stephens).

0042-6822/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2010.07.021

proteins to counteract BST-2 restriction is species-specific (Gupta et al., 2009; Jia et al., 2009; McNatt et al., 2009; Zhang et al., 2009). Specific domains and amino acids within these proteins mediate this specificity, allowing small evolutionary changes to confer susceptibility to cross-species infection (Gupta et al., 2009; Jia et al., 2009; McNatt et al., 2009; Yap et al., 2005; Zhang et al., 2009). The interaction of Vpu with hBST-2 involves the TMDs of both proteins (Douglas et al., 2009; McNatt et al., 2009; Rong et al., 2009). Several studies have identified amino acids within the human, rhesus and tantalus monkey BST-2 proteins required for Vpu sensitivity/resistance. These studies found several combinations of substitutions within the transmembrane domain of hBST-2 that reduced susceptibility to Vpu, including delGI,T45I (where glycine and isoleucine residues at positions 25 and 26 were deleted in combination with the substitution of a threonine at position 45 with an isoleucine) and delGI, I33V, I36L (where the glycine and isoleucine residues at positions 25 and 26 were deleted in combination with the substitution of the isoleucine at position 33 with a valine and mutation of the isoleucine at position 36 to a leucine). These studies also noted the importance of the proline residue at position 40 in sensitivity to Vpu (McNatt et al., 2009). More recent studies focused on the specific role of amino acids within the BST-2 proteins from non-human primates in the susceptibility and resistance to SIV_{mac} Nef proteins. Several groups obtained similar results that identified an amino acid motif that is present within the cytoplasmic domain ($D/GIWK_{14–17}$) of pig-tailed and rhBST-2 as important for SIV Nef susceptibility. These investigators showed that this motif could be inserted into the hBST-2 protein and impart susceptibility to the SIV Nef protein (Jia et al., 2009; Zhang et al., 2009).

Simian–human immunodeficiency viruses (SHIVs) have been used extensively for *in vivo* studies to understand the role of HIV-1 Vpu and Env proteins as well as other SIV proteins in viral pathogenesis. These chimeric viruses express both the HIV-1 Vpu and SIV_{mac} Nef proteins shown to counteract BST-2 orthologues from human and non-human primates and possibly represent a useful virus to study these interactions. In this study, we examined the characteristics and anti-viral activities of BST-2 proteins from humans and pig-tailed macaques with specific amino acid exchanges in the context of SHIVs lacking the HIV-1 *vpu* gene, the SIV *nef* gene, or both. Our results indicate that the transmembrane domain is a determinant of HIV-1 Vpu susceptibility, however, the length of the domain may play more of a role in this rather than specific amino acids and or combinations of residues.

Results

Comparison of the sequence of pig-tailed and rhesus bst-2 genes

We amplified and sequenced the *bst-2* genes from five rhesus macaques (CX54, AH64, AS05, AS34 and AS89) and seven pig-tailed macaques (CC8X, W004, W005, W006, W007, W013, and W018). The genes isolated from the rhesus macaques exhibited considerable variability compared to genes isolated from pig-tailed macaques, most notably in the N-terminal regions (Fig. 1). We observed a 50:50 distribution of arginine and cysteine at position 9, a 60:40 ratio of aspartic acid to glycine at position 14, a 70:30 distribution of valine to isoleucine at position 29 and finally an 80:20 ratio of leucine to proline at position 43. In contrast the pig-tailed sequences did not show significant variability with the exception of one sequence that had a leucine instead of a methionine at position 13. Based on the sequence analysis, we cloned the *ptbst-2* gene for expression studies.

Analysis of human and ptBST-2 mutants

We constructed a series of mutations in the human (hBST-2) and pig-tailed macaque (ptBST-2) *bst-2* genes. These are presented in Fig. 2. We first examined the expression of each mutant BST-2 protein by transfection of 293 cells with vectors expressing each mutant protein and subsequent Western blot analysis of the cell lysates (Fig. 3). Substitution of the leucine and glycine residues at positions 24 and 25 in hBST-2 with alanines or isoleucines (hBST-2LG/AA or hBST-2LG/II) consistently resulted in higher levels of expression, suggesting that these mutations may increase the half-life of these proteins. The only other protein with altered expression was the ptBST-2(ΔDDWIK/+LG). This amino acid substitution resulted in decreased protein expression, which could be due to altered stability of the protein and was not further studied.

SHIV can serve as models for studying BST-2 mediated restriction

In order to examine the ability of the wild-type hBST-2 and ptBST-2 proteins to restrict virus release, we used parental $SHIV_{KU-2MC4}$ to construct three SHIVs that did not express either HIV-1 Vpu ($SHIV_{\Delta Vpu}$), SIV Nef ($SHIV_{\Delta Nef}$) or both proteins ($SHIV_{\Delta Vpu/\Delta Nef}$). HEK 293 cells were co-transfected with plasmids expressing one of the four SHIVs and each of the BST-2 constructs. At 48 h post-transfection, the supernatants were collected and cleared by centrifugation, the cells were lysed and the nuclei and cellular debris cleared by centrifugation. The supernatants and cell lysates were used to quantify the percent p27 antigen release from cells and the supernatants were also evaluated for the number of infectious doses ($TCID_{50}$) (Fig. 4). The percent release of p27 in the context of each SHIV in the presence and absence of each BST-2 protein is shown in Fig. 4A. All samples were normalized to their respective SHIV empty vector controls. Significance in the restriction of p27 release for the $SHIV_{KU-2MC4}$ samples was determined with respect to the parental $SHIV_{KU-2MC4}$ empty vector control using a Student's t-test (▲). Significance in the restriction of p27 release for the $SHIV_{\Delta Vpu}$, $SHIV_{\Delta Nef}$, and $SHIV_{\Delta Vpu/\Delta Nef}$ samples was calculated with respect to the $SHIV_{KU-2MC4}$ in the presence of each respective BST-2 using a Student's t-test (*). The level of infectious virus released was determined using TZM-bl indicator cells (Fig. 4B). The results are represented as the average $\log_{10}$ infectious units. Significance in the restriction of infectious units released was determined with respect to the appropriate empty vector control (•) since Nef has significant effects on virion infectivity that are not associated with BST-2 incorporation (Aiken and Trono, 1995; Chowers et al., 1994). All four SHIVs exhibited similar p27 release in the absence of any BST-2 protein. In the presence of hBST-2, $SHIV_{\Delta Vpu}$ and $SHIV_{\Delta Vpu/\Delta Nef}$ viruses exhibited a significant decrease in p27 release from cells while $SHIV_{\Delta Nef}$ had no decrease in p27 release. Similar results were observed in the infectious units assay. In the presence of ptBST-2 protein, only $SHIV_{\Delta Nef}$ and $SHIV_{\Delta Vpu/\Delta Nef}$ showed a decrease in p27 release as well as infectious units released. These results are in congruence with those published by other investigators that found that hBST-2 is susceptible to HIV-1 Vpu and ptBST-2 is sensitive to the SIV Nef (Neil et al., 2008; Van Damme et al., 2008; Zhang et al., 2009), indicating that these SHIVs can serve as models for studying the individual and additive effects of different BST-2 proteins.

Mutant ptBST-2 antagonism of SHIV release

Previous studies showed that the transmembrane domain of the hBST-2 protein dictates its interaction with and susceptibility to HIV-1 Vpu (McNatt et al., 2009; Gupta et al., 2009; Rong et al., 2009). We determined if cumulative consecutive amino acid substitutions in the transmembrane domain of the ptBST-2 would result in a gradual increase in sensitivity to Vpu. We generated a library of ptBST-2 constructs with successive “humanizing” amino acid substitutions starting from the amino terminal end of the TMD with an insertion of two amino acids, a leucine and a glycine, at positions 29 and 30. We transfected each construct into 293 cells in conjunction with one of four different simian–human immunodeficiency virus expressing plasmids. The supernatants and cell lysates were used to quantify the percent p27 antigen release from cells and the supernatants were also evaluated for the number of infectious