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Substitution of the transmembrane domain of Vpu in simian–human immunodeficiency virus ($SHIV_{KU1bMC33}$) with that of M2 of influenza A results in a virus that is sensitive to inhibitors of the M2 ion channel and is pathogenic for pig-tailed macaques

David R. Hout [a], Melissa L. Gomez [a], Erik Pacyniak [a], Lisa M. Gomez [a], Barbara Fegley [a], Ellyn R. Mulcahy [a], M. Sarah Hill [a], Nathan Culley [b], David M. Pinson [c], Warren Nothnick [d], Michael F. Powers [e], Scott W. Wong [e], Edward B. Stephens [a,*]

[a] *Department of Anatomy and Cell Biology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, USA*
[b] *Laboratory Animal Resources, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, USA*
[c] *Department of Laboratory Medicine and Pathology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, USA*
[d] *Department of Obstetrics and Gynecology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, USA*
[e] *Vaccine and Gene Therapy Institute, Oregon National Primate Research Center, Oregon University for the Health Sciences, Beaverton, OR 97003, USA*

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Abstract

The Vpu protein of human immunodeficiency virus type 1 has been shown to shunt the CD4 receptor molecule to the proteasome for degradation and to enhance virus release from infected cells. The exact mechanism by which the Vpu protein enhances virus release is currently unknown but some investigators have shown that this function is associated with the transmembrane domain and potential ion channel properties. In this study, we determined if the transmembrane domain of Vpu could be functionally substituted with that of the prototypical viroporin, the M2 protein of influenza A virus. We constructed chimeric *vpu* gene in which the transmembrane domain of Vpu was replaced with that of the M2 protein of influenza. This chimeric *vpu* gene was substituted for the *vpu* gene in the genome of a pathogenic simian human immunodeficiency virus, $SHIV_{KU-1bMC33}$. The resulting virus, $SHIV_{M2}$, synthesized a Vpu protein that had a slightly different M_r compared to the parental $SHIV_{KU-1bMC33}$, reflecting the different sizes of the two Vpu proteins. The $SHIV_{M2}$ was shown to replicate with slightly reduced kinetics when compared to the parental $SHIV_{KU-1bMC33}$ but electron microscopy revealed that the site of maturation was similar to the parental virus $SHIV_{KU1bMC33}$. We show that the replication and spread of $SHIV_{M2}$ could be blocked with the antiviral drug rimantadine, which is known to target the M2 ion channel. Our results indicate a dose dependent inhibition of $SHIV_{M2}$ with 100 μM rimantadine resulting in a >95% decrease in p27 released into the culture medium. Rimantadine did not affect the replication of the parental $SHIV_{KU-1bMC33}$. Examination of $SHIV_{M2}$-infected cells treated with 50 μM rimantadine revealed numerous viral particles associated with the cell plasma membrane and within intracytoplasmic vesicles, which is similar to HIV-1 mutants lacking a functional *vpu*. To determine if $SHIV_{M2}$ was as pathogenic as the parental $SHIV_{KU-1bMC33}$ virus, two pig-tailed macaques were inoculated and followed for up to 8 months. Both pig-tailed macaques developed severe $CD4^+$ T cell loss within 1 month of inoculation, high viral loads, and histological lesions consistent with lymphoid depletion similar to the parental $SHIV_{KU-1bMC33}$. Taken together, these results indicate for the first time that the TM domain of the Vpu protein can be functionally substituted with the TM of M2 of influenza A virus, and shows that compounds that target the TM domain of Vpu protein of HIV-1 could serve as novel anti-HIV-1 drugs.

Keywords: Vpu; M2 protein; Viroporin; SHIV; Pathogenesis

Introduction

Human immunodeficiency virus type 1 (HIV-1) and several strains of simian immunodeficiency viruses (SIV) isolated from

* Corresponding author. Fax: +1 913 588 2710.
E-mail address: estephen@kumc.edu (E.B. Stephens).

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Fig. 1. Sequence of the proteins analyzed in this study. The (−) introduced in the Vpu_{M2} sequence was for purposes of sequence alignment.

non-human primates encode for a small membrane bound protein known as the Vpu protein (Strebel et al., 1988; Cohen et al., 1988; Huet et al., 1990; Barlow et al., 2003; Courgnaud et al., 2002; 2003). The Vpu protein from laboratory-adapted subtype B HIV-1 has been extensively studied with respect to its role in the virus life cycle and has resulted in the identification of two major functions in replication. The Vpu down-regulates the CD4 receptor in the rough endoplasmic reticulum (RER) and shunts it to the proteasome for degradation (Fujita et al., 1997; Schubert et al., 1998). Studies have shown that the highly conserved hinge region containing two casein kinase II sites is required for the CD4 degradation (Schubert et al., 1994; Paul and Jabbar, 1997) and other studies have shown that the predicted two α-helical domains within the cytoplasmic domain and sequences within the transmembrane (TM) domain are also required for efficient degradation of CD4 (Tiganos et al., 1998). Additionally, Vpu also enhances virus release from infected cells (Klimkait et al., 1990). Examination of cells infected with HIV-1 viruses containing large deletions within the *vpu* gene by electron microscopy revealed a different pattern of virus maturation with many particles tethered together at the cell surface and within intracellular vesicles. Some investigators have mapped Vpu-mediated virion release to the TM domain and other investigators have provided evidence that the TM domain forms an ion channel (Ewart et al., 1996; Schubert et al., 1996a). This evidence suggests that Vpu of HIV-1 is a member of a class of viral proteins known as the viroporins, which include the M2 protein of influenza A virus, the 6K protein of Sindbis virus (SV), and the 2B protein of poliovirus (Gonzalez and Carrasco, 2003).

In a previous study, evidence was provided that one viroporin could substitute for one another (Gonzalez and Carrasco, 2001).

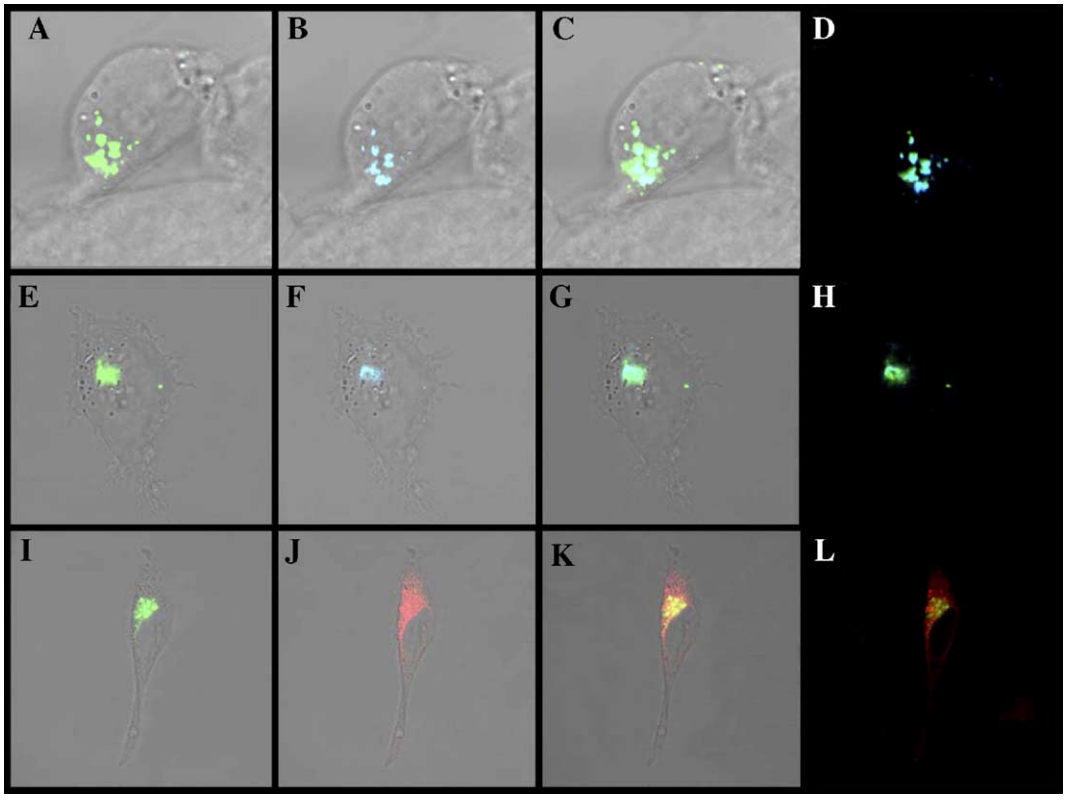

Fig. 2. Vpu_{M2}EGFP is expressed in the same intracellular compartments as the wild type Vpu. 293 cells were co-transfected with vectors expressing VpuEGFP or Vpu_{M2}EGFP and DsRed2-ER or ECFP-Golgi. At 48 h, cells expressing EGFP and DsRed2 or ECFP were identified and images collected using laser scanning confocal microscopy as described in the Materials and methods section. (A–D) 293 cells co-transfected with VpuEGFP and ECFP-Golgi. (A) Expression of VpuEGFP. (B) Expression of ECFP-Golgi. (C) Merge of panels A and B. (D) Fluorescence micrograph of EGFP and ECFP fusion proteins from panels A and B. (E–H) 293 cells co-transfected with Vpu_{M2}EGFP and ECFP-Golgi. (E) Expression of Vpu_{M2}EGFP. (F) Expression of ECFP-Golgi. (G) Merge of panels E and F. (H) Fluorescence micrograph of EGFP and ECFP fusion proteins from panels E and F. (I–L) 293 cells co-transfected with Vpu_{M2}EGFP and DsRed2-ER. (I) Expression of Vpu_{M2}EGFP. (J) Expression of DsRed2-ER. (K) Merge of panels I and J. (L) Fluorescence micrograph of EGFP and DsRed2 fusion proteins from panels I and J.