Enhanced antagonism of BST-2 by neurovirulent SIV envelope

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Introduction

Although neuroAIDS or AIDS dementia caused by HIV-1 has been declining in clinical practice due to the use of Highly Active Antiretroviral Therapy (HAART), a milder neurologic disorder termed Minor Cognitive Motor Disorder (MCMD) continues to be a significant clinical problem.

To develop new strategies to intervene in HIV disease progression in the brain, our lab has been developing a novel nonhuman primate neuroAIDS model by in vivo passage of SIVsmE543-3, a strain that is capable of inducing AIDS in the infected rhesus macaques but not SIV encephalitis (SIVE). As a result, we have recently isolated a viral strain, SIVsm804E, which can cause neuroAIDS in infected animals at high frequencies (Matsuda et al., JVI 2013, Matsuda et al., JVI 2014). A molecular clone, SIVsm804E CL757 was then isolated from the SIVsm804E viral swarm. Introduction of the gp41 cytoplasmic tail of Env of this molecular clone into the parental SIVsmE543-3 backbone, resulted in improvement of viral replication in monocyte derived macrophages (MDMs) in vitro.

It has been previously reported that the cytoplasmic tail of gp41 of SIV plays an important role in the antagonism of the host factor, bone marrow stromal antigen 2 (BST-2, also known as Tetherin), that impairs the release of nascent viral particles from the virus-producing cells (Serra-Moreno et al., Cell host microbes 2011).

To determine whether efficient antagonism of BST-2 is responsible for the improvement of replication in macrophages, pulse-chase and immmunoprecipitation assays were conducted to compare the efficacy of Tetherin antagonism by the original SIVsmE543 to that of a variant with the cytoplasmic tail mutations found in SIVsm804E CL757.

RESULTS

Enhanced replication in MDM by introduction of gp41/nef/LTR of SIVsm804E to SIVsmE543-3

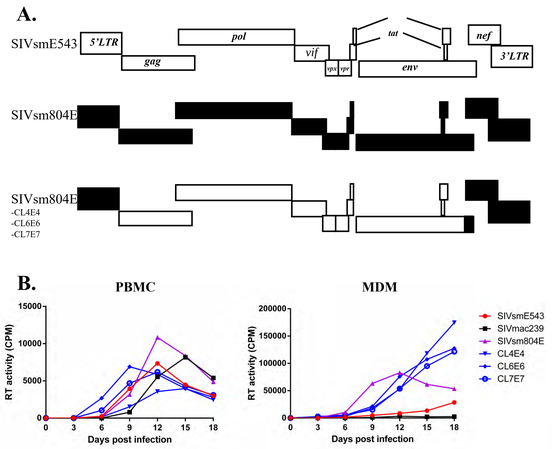


Figure 1. Constructs used in the current study. (A) Schematic representation of the SIVsm804E constructs. (C) PBMC and MDMs were inoculated with each virus at an MOI of 0.01. Virus replication was monitored by measuring the virus-associated RT activity in the culture supernatants over time.

12 mutations were consistent in all three SIVsm804E clones

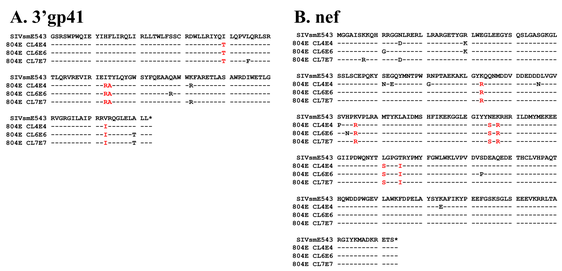


Figure 2. Amino acid alignment of SIVsm804E clones. (A) 3' end of gp41 alignment. (B) nef alignment.

Introduction of the gp41 portion of 804E into the SIVsmE543-3 backbone enhances viral production from infected MDMs

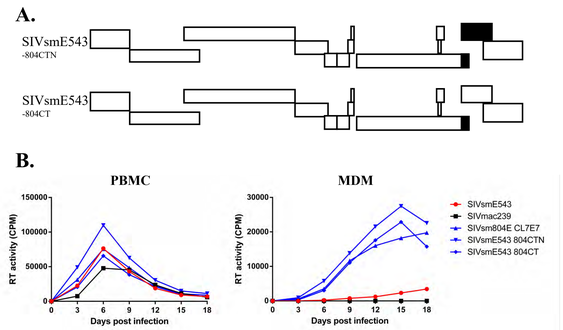


Figure 3. The Nef/gp41 or gp41 portion of 804E was introduced to SIVsmE543-3 backbone that enhances viral replication in macrophages. (A) The Nef/gp41 or gp41 portion of SIVsm804E was introduced into the SIVsmE543-3 backbone. (B) Replication kinetics of SIVsmE543-804CTN and 804CT.

Mutations in the C-terminal part of gp41 cytoplasmic tail contribute to enhanced replication of SIVsmE543 in macrophages

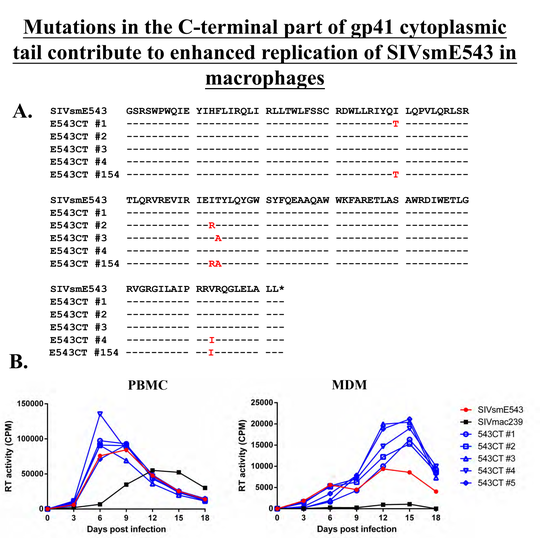


Figure 4. Mutations in the cytoplasmic tail of SIVsmm gp41 enhanced replication of SIVsmE543 in macrophages. (A) Mutations were introduced to original SIVsmE543 (B) Replication kinetics of SIVsmE543 mutants in PBMC and MDM.

Mutations do nod affect amount of virion incorporated envelope protein

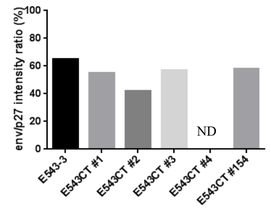


Figure 5. Mutations in the cytoplasmic tail of SIVsmm gp41 did not affect the level of virion incorporated envelope protein. Envelope protein level of mutants were compared with original SIVsmE543 by western blotting normalizing with p27 level.

SIVs antagonize BST-2 by envelope protein

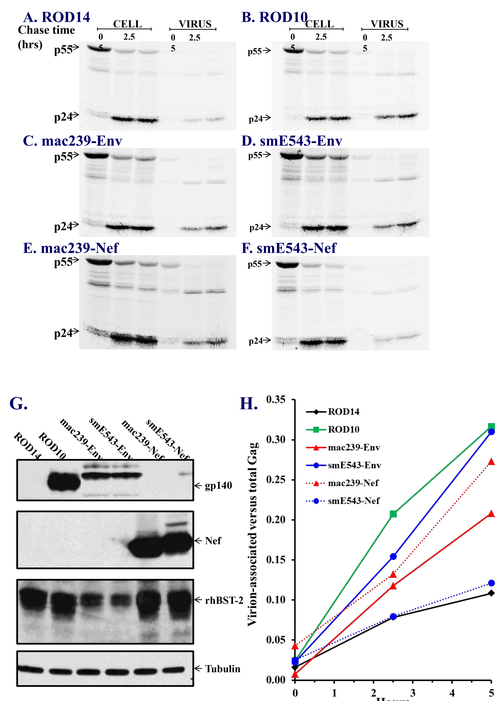


Figure 5. The SIVsmm envelope glycoprotein enhances HIV-1 particle release. (A to F) Kinetic analysis of viral particle release by the Vpu-deficient pNL4-3/Udel-1 in the HIV-2 envelope protein, SIV envelope protein and SIV Nef protein. 293T cells were transfected with pNL4-3/Udel-1 and rhBST-2 DNA together with HIV-2 Env vectors pCM14-Env (A) and pHA-ROD10-Env (B) as controls, as well as vectors for the expression of HA-tagged Envs from SIVmac239 (C) and SIVsmE543 (D) , or vectors for the expression of HA-tagged Nef from SIVmac239 (E) and SIVsmE543 (F). Samples were subjected to pulse-chase analysis and viral proteins recovered by immunoprecipitation were separated by SDS–PAGE. The HIV-1 major Gag proteins p55*gag* and p24CA are identified on the left. (G) Protein expression for Envelope, Nef and rhBST-2 was verified by western blot analysis using cellular α-tubulin as a loading control. (H) Bands corresponding to the precursor and mature Gag proteins in panels A through F were quantified and the efficiency of particle release at each time point was calculated and plotted as a function of time.

Mutations in the cytoplasmic tail of SIVsmm gp41 enhance viral particle release

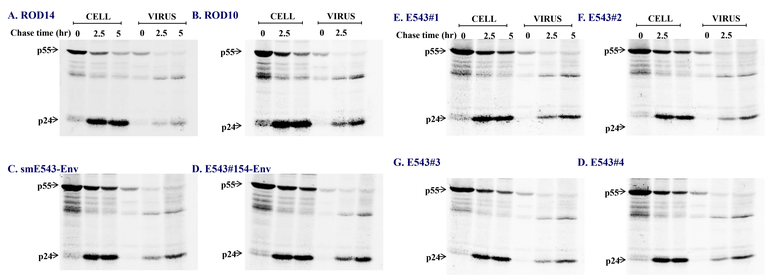


Figure 6. Effect of cytoplasmic tail mutations of SIVsmm gp41 on HIV-1Δ*vpu* particle release efficiency. (A) (B to E) Kinetic analysis of viral particle release by SIVsmE543 cytoplasmic tail mutants. HeLa cells were transfected with pNL4-3/Udel together with vectors expressing HA-tagged Envs from SIVsmE543 wt, or cytoplasmic tail mutants. Cells were pulse-chased and viral proteins recovered by immunoprecipitation were separated by SDS-PAGE. HIV-1 Gag products p55*gag* and p24CA are identified on the left.

Mutations were accumulated during sequential passages through the brain.

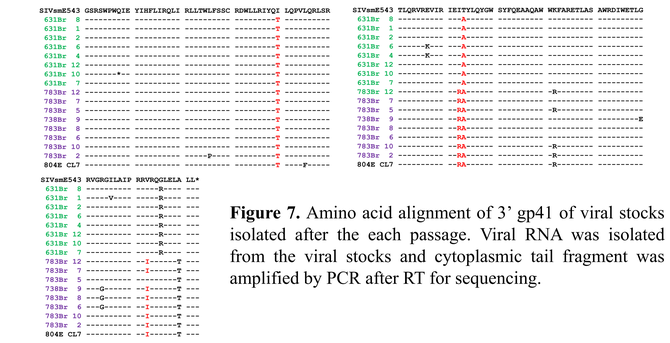


Figure 7. Amino acid alignment of 3' gp41 of viral stocks isolated after the each passage. Viral RNA was isolated from the viral stocks and cytoplasmic tail fragment was amplified by PCR after RT for sequencing.

Conclusion

Sequential *in vivo* passages of SIVsmE543 conferred enhanced replication in macrophages. This enhancement was associated with the mutations at the cytoplasmic tail of gp41 but did not increase the level of virion associated envelope protein as it has previously been reported. Instead, our data show that multiple mutations in the cytoplasmic tail of gp41 individually enhance viral release from the cells expressing BST-2.

Since macrophages are the main target for viral infection and replication in the brain of SIV infected animals, our results suggest that our virus accumulated mutations in the cytoplasmic tail of gp41 through the sequential passages for efficient replication in the central nervous system (CNS).

Our data also show that SIV uses different protein to antagonize BST-2, even among the closely related strains which shares the common ancestor. This observation is interesting in evolutionally aspect since HIV-2, which came from SIVsm, thought to be only virus uses it's envelope to antagonize BST-2 but our data show some SIV strains uses envelope too, instead of nef protein.

Although SIV and HIV-1 utilize different viral proteins to antagonize BST-2, our data suggest that BST-2 may play an important role in the suppression of viral replication in the CNS and intervention of viral factors that antagonize BST-2 could be a novel strategy for prevention of disease progression in the CNS during HAART.

ACKNOWLEDGEMENT

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