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Lentivirus restriction by diverse primate APOBEC3A proteins

Kimberly Schmitt [a], Kejun Guo [b], Miki Katuwal [a], Darayu Wilson [a], Courtney Prochnow [c], Ronda Bransteitter [c], Xiaojiang S. Chen [c], Mario L. Santiago [b,*], Edward B. Stephens [a,*]

[a] Department of Microbiology, Molecular Genetics, and Immunology, University of Kansas Medical Center, 3901 Rainbow Blvd. Kansas City, KS 66160, United States
[b] Department of Medicine, Microbiology and Immunology, University of Colorado Denver School of Medicine, Aurora, CO 80045, United States
[c] Department of Molecular and Computational Biology, University of Southern California, CA 90089, United States

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ABSTRACT

Rhesus macaque APOBEC3A (rhA3A) is capable of restricting both simian–human immunodeficiency virus (SHIVΔ*vif*) and human immunodeficiency virus (HIV-1Δ*vif*) to a greater extent than hA3A. We constructed chimeric A3A proteins to define the domains required for differential lentivirus restriction. Substitution of amino acids 25–33 from rhA3A into hA3A was sufficient to restrict HIVΔ*vif* to levels similar to rhA3A restriction of SHIVΔ*vif*. We tested if differential lentivirus restriction is conserved between A3A from Old World monkey and hominid lineages. A3A from African green monkey restricted SHIVΔ*vif* but not HIV-1Δ*vif* and colobus monkey A3A restricted both wild type and SHIVΔ*vif* and HIV-1Δ*vif*. In contrast, the gibbon ape A3A restricted neither SHIVΔ*vif* nor HIV-1Δ*vif*. Restriction of SHIVΔ*vif* and HIV-1Δ*vif* by New World monkey A3A proteins was not conserved as the A3A from the squirrel monkey but not the northern owl monkey restricted SHIVΔ*vif*. Finally, the colobus A3A protein appears to restrict by a novel post-entry mechanism.

Introduction

The apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3 (APOBEC3; A3) family of proteins in humans have been shown to restrict the replication of many different retroviruses (Sheehy et al., 2002; Chiu and Greene, 2009). In humans and rhesus macaques, this family consists of seven members (A3A, A3B, A3C, A3D, A3F, A3G and A3H) (Dang et al., 2007; Hultquist et al., 2011; Jarmuz et al., 2002; Schmitt et al., 2011; Virgen et al., 2007). These proteins are cytidine deaminases that have canonical deaminase domains (H-x-E-x_{24-28}-P-C-x-x-C) where the histidine and two cysteines coordinate a zinc cofactor, with the glutamate serving in proton transfer (MacGinnitie et al., 1995). The A3 proteins contain either a single cytidine deaminase domain (A3A, A3C, A3H) or duplicated domains (A3B, A3D, A3F, and A3G). Following entry of virus containing A3G, this enzyme causes deamination of cytidine to uridine during minus strand DNA synthesis, ultimately resulting in G-to-A mutations (Chelico et al., 2006; Yu et al., 2004).

HIV-1 has evolved a Vif protein to counter incorporation of select A3 proteins during virus maturation by interacting with and shunting these proteins to the proteasome via the Cul5/ElonginB/C/rbx E3 ubiquitin ligase (Liu et al., 2004; Marin et al., 2003; Mehle et al., 2004; Sheehy et al., 2003; Yu et al., 2003). Of the A3 proteins, the double deaminase domain proteins (A3B, A3D, A3F, and A3G) are most often associated with the restriction of HIVΔ*vif* (Dang et al., 2007; Doehle et al., 2005; Sheehy et al., 2002; Wiegand et al., 2004). Human A3C, a single deaminase domain protein, is incorporated into both HIV-1 and HIV-1Δ*vif* and is capable of inducing G-to-A mutations in the viral genome, although not to the same extent as A3G (Bourara et al., 2007; Kitamura et al., 2010; Langlois et al., 2005; Smith et al., 2010; Wang et al., 2008). Investigators have found that alleic differences of hA3H accounts for the ability of select hA3H proteins to potently inhibit HIV-1Δ*vif* (Harari et al., 2009; Li et al., 2010; OhAinle et al., 2008; Ooms et al., 2010; Wang et al., 2011).

Virus restriction experiments on hA3A performed in epithelial cell lines such as HeLa or 293 cells indicate that hA3A does not inhibit HIV-1Δ*vif* (Aguiar et al., 2008; Bishop et al., 2004; Goila-Gaur et al., 2007). However, hA3A can restrict parvoviruses and LINE-1 elements (Babushok et al., 2007; Bogerd et al., 2006a, 2006b; Brouha et al., 2003; Muckenfuss et al., 2006). Other studies showed that hA3A can inhibit the replication of adeno-associated virus type 2 (AAV-2) through a deaminase-independent mechanism, can activate the DNA damage response, cause preferential degradation of plasmid DNA, and inhibit human T-cell leukemia virus type 1 (HTLV-I) (Chen et al., 2006; Landry et al., 2011; Narvaiza et al., 2009; Ooms et al., 2012; Stenglein et al., 2010).

* Corresponding authors. Fax: +1 913 588 7295.
E-mail address: estephen@kumc.edu (E.B. Stephens).

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Recently, we showed that rhesus macaques also express an A3A protein (rhA3A) that restricted *vif*-deleted SHIV and to a lesser extent HIV-1Δ*vif* (Schmitt et al., 2011). We showed that a three amino acid deletion in hA3A was partially responsible for the lack of restriction. Currently, we do not know whether the differential ability of rhA3A and hA3A to restrict lentiviruses is a conserved property of the corresponding Old World monkey (OWM) and hominid A3A lineages, respectively. Additionally, no information is available on whether A3A from New World monkeys (NWM), which are not known have endemic lentiviruses, are capable of restricting HIV-1 or SIV/SHIV. In this study, we further define the amino acid deletions/substitutions necessary to restore the virus restriction activity of hA3A. We show that the A3A from one NWM is capable of restricting SHIV. Finally, we present data that one OWM A3A protein from *Colobus guereza* can inhibit lentiviral replication by novel mechanism.

Results

The location of the HA-tag does not effect subcellular location

We determined if fusion of the HA-tag to the N-terminus or C-terminus of rhA3A affected the nucleocytoplasmic localization of the protein and restriction of SHIV. 293 cells were transfected with vectors expressing HA-rhA3G, HA-hA3A, hA3A-HA, HA-rhA3A, rhA3A-HA, and rhA3A-3XHA. At 48 h, cells were starved, radiolabeled for 1 h. and used in immunoprecipitation assays using an antibody against the HA-tag. As shown in Fig. 1, all A3A proteins were expressed as doublets, which was previously shown to be due the initiation of translation at an alternative methionine at position 12 (Theilen et al., 2010). Interestingly, placement of the HA-tag at the amino terminus of rhA3A (HA-rhA3A) resulted in a slight shift in the mobility compared to the rhA3A-HA. Previous studies have shown that hA3G is localized in the cytoplasm while rhA3A is found in both the cytoplasm and nucleus (Bogerd et al., 2006a,2006b; Chen et al., 2006; Goila-Gaur et al., 2007; Muckenfuss et al., 2006; Schmitt et al., 2011). We examined the intracellular localization of the HA-tagged A3A proteins using immunofluorescence and confocal microscopy. We found that HA-rhA3G was observed exclusively in the cytoplasm as previously reported (Fig. 2) while HA-rhA3A, rhA3A-HA, and rhA3A-3XHA were detected in both the cytoplasm and nucleus as it co-localized with a eGFP-tagged nuclear marker (Fig. 2). Thus, any differences in the retroviral restriction properties of rhA3A proteins could not be explained by the changes in steady-state levels of the proteins or subcellular localization.

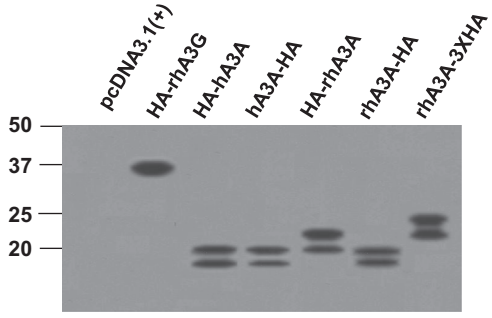

Fig. 1. Expression of HA-rhA3G, hA3A-HA, HA-rhA3A, rhA3A-HA, and rhA3A-3XHA. Panel A. 293 cells were transfected with vectors expressing the proteins listed above. At 48 h, the cells were starved for methionine/cysteine and radiolabeled with ^{35}S-methionine/cysteine for 1 h, cells lysed in 1X RIPA buffer and HA containing proteins immunoprecipitated using an anti-HA serum as described in the Materials and methods section.

Virus restriction of the HA-tagged rhesus and human A3A proteins

We next determined if fusion of the HA-tag to the N- or C-terminus of rhA3A affected the restriction of SHIV. For virus restriction assays, 293 cells were transfected with vectors containing the SHIVΔ*vif* or SHIV$_{KU-2MC4}$ genomes and vectors expressing either HA-hA3A, hA3A-HA, HA-rhA3A, rhA3A-HA, rhA3A-3xHA, untagged rhA3A or vector alone. At 48 h the culture supernatants were collected and the infectious titers of the released viruses determined. As shown in Fig. 3, fusion of the HA-tag to the N-terminus of rhA3A resulted in approximately a 20-fold reduction in virus infectivity, which was similar to the results with the untagged rhA3A (Fig. 3). Fusion of a single HA-tag or a 3X HA-tag to the C-terminus of rhA3A resulted in a reduction of virus infectivity by 2–5-fold and 1–2-fold, respectively. All experiments were performed in triplicate and significance in the restriction of infectious SHIV$_{KU-2MC4}$ or SHIVΔ*vif* was calculated with respect to the empty vector control using a Student's two-tailed *t*-test (*). The reduction in infectivity was found to be significant for the HA-rhA3A and untagged rhA3A but not significant for the rhA3A-HA or rhA3A-3XHA. From these results, we conclude that HA-rhA3A more closely reflects the native rhA3A with respect to restriction of lentiviruses.

The N-terminal half of the rhA3A contains the necessary determinants for virus restriction

We previously showed that a 3 amino-acid indel (^{27}SVR^{29} in rhA3A) is important for differential lentivirus restriction between rhA3A and hA3A (Schmitt et al., 2011). To determine the domains of rhA3A that could completely restore hA3A lentivirus restriction, we constructed a series of chimeric rhesus/human A3A proteins and evaluated their restriction properties against SHIVΔ*vif* and HIV-1Δ*vif* (Fig. 4). We analyzed the stability of these proteins by transfection of 293 cells with vectors expressing each chimeric protein or HA-rhA3A. At 48 h, transfected cells were radiolabeled for one hour and the HA-tagged A3 proteins immunoprecipitated with an anti-HA antibody as described in Fig. 1. Our results indicate that these chimeric proteins were readily expressed in 293 cells (Fig. 5). However, these chimeric proteins had divergent activities on SHIV infectivity with the HA-rh$_{1-100}$hA3A efficiently restricting infectivity of SHIVΔ*vif* and HIV-1Δ*vif* while the HA-h$_{1-100}$rhA3A did not restrict SHIVΔ*vif* or HIVΔ*vif* infectivity (Fig. 6A and B). From these results we conclude that N-terminal half of the rhA3A had the necessary amino acid substitutions for restriction of SHIVΔ*vif* and HIV-1Δ*vif*. We next analyzed chimeric proteins in which amino acids 1–33, 1–50 and 16–50 amino acids of hA3A were replaced with the analogous region from rhA3A (rh$_{1-33}$hA3A, rh$_{1-50}$hA3A, and rh$_{16-50}$hA3A, respectively). When analyzed in restriction assays, rh$_{1-33}$hA3A, rh$_{1-50}$hA3A, and rh$_{16-50}$hA3A all restricted the replication of SHIVΔ*vif* and HIV-1Δ*vif* (Fig. 6A and B). We further narrowed the amino acid changes required with another chimeric protein in which amino acids 25–33 of rhA3A were substituted into the same region of hA3A (rh$_{25-33}$hA3A). Our analysis revealed that the rh$_{25-33}$hA3A had a similar virus restriction profile to rhA3A (Fig. 6A and B). From these studies we conclude that the six amino acid substitutions in this region of rhA3A were sufficient to completely restore hA3A restriction activity against SHIVΔ*vif* and HIV-1Δ*vif*.

We also determined if rh$_{25-33}$hA3A was incorporated in viral nucleocapsids. Previous studies have shown that hA3G but not hA3A was incorporated into viral nucleocapsids (Aguiar et al., 2008; Goila-Gaur et al., 2007; Schmitt et al., 2011). For these experiments, 293 cells were co-transfected with plasmids with either the HIV-1 or HIV-1Δ*vif* genome and plasmids that expressed