JOURNAL OF VIROLOGY, June 2006, p. 5984–5991 Vol. 80, No. 12
0022-538X/06/$08.00+0 doi:10.1128/JVI.00388-06

Mutational Alteration of Human Immunodeficiency Virus Type 1 Vif Allows for Functional Interaction with Nonhuman Primate APOBEC3G†

Bärbel Schröfelbauer,[1,3] Tilo Senger,[1,4] Gerard Manning,[2] and Nathaniel R. Landau[1]*

Infectious Disease Laboratory[1] and Newman Center for Bioinformatics,[2] The Salk Institute for Biological Studies, La Jolla, California 92037; Department of Biotechnology, Institute of Applied Microbiology, University of Natural Resources and Applied Life Sciences, A-1180 Vienna, Austria[3]; and Technical University Carolo-Wilhelmina of Braunschweig, Braunschweig, Germany[4]

Received 23 February 2006/Accepted 5 April 2006

Human APOBEC3F (hA3F) and APOBEC3G (hA3G) are antiretroviral cytidine deaminases that can be encapsidated during virus assembly to catalyze C→U deamination of the viral reverse transcripts in the next round of infection. Lentiviruses such as human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) have evolved the accessory protein Vif to induce their degradation before packaging. HIV type 1 (HIV-1) Vif counteracts hA3G but not rhesus macaque APOBEC3G (rhA3G) or African green monkey (AGM) APOBEC3G (agmA3G) because of a failure to bind the nonhuman primate proteins. The species specificity of the interaction is controlled by amino acid 128, which is aspartate in hA3G and lysine in rhA3G. With the objective of overcoming this species restriction, mutations were introduced into HIV-1 Vif at amino acid positions that differed in charge between HIV-1 Vif and SIV Vif. The mutant proteins were tested for the ability to counteract hA3G, rhA3G, and agmA3G. Alteration of the conserved sequence at positions 14 to 17 from DRMR to SERQ, which is the sequence in AGM Vif, caused HIV-1 Vif to functionally interact with rhA3G and agmA3G. Mutation of three residues to the sequence SEMQ allowed interaction with rhA3G. SEMQ Vif also counteracted D128K mutant hA3G and wild-type hA3G. Introduction of the sequence into an infectious molecular HIV-1 clone allowed the virus to replicate productively in human cells that expressed rhA3G or hA3G. These findings provide insight into the interaction of Vif with A3G and are a step toward the development of a novel primate model for AIDS.

Several nonhuman primate models for AIDS have been used to provide insight into human immunodeficiency virus (HIV) pathogenesis in humans. In particular, simian immunodeficiency virus of macaques (SIVmac) and chimeric SIV-HIV induce AIDS-like symptoms in rhesus macaques through pathogenic mechanisms that mimic those of HIV type 1 (HIV-1) (7). HIV-1, however, does not infect rhesus macaques or nonhuman primates such as the African green monkey (AGM) (1, 10–12, 19, 23). This species restriction is caused by at least two blocks to HIV-1 replication in nonhuman primate T cells and macrophages: a defect in the particle infectivity of virions that are released which is mediated by host APOBEC3 proteins and a postentry block that is mediated by Trim5α (25).

Lentiviruses such as HIV-1 and SIV encode Vif, an accessory protein that counteracts the antiviral activity of the APOBEC3 family of cytidine deaminases (9). Human APOBEC3G (hA3G) was identified initially (22), and subsequently additional family members, including human APOBEC3B, human APOBEC3C, and human APOBEC3F (hA3F), were found to be active (14, 27, 34). In cells infected with HIV-1 with *vif* deleted (Δ*vif*), hA3G and hA3F are packaged into assembling virions. In the next round of infection, the packaged enzymes deaminate the newly synthesized minus-strand cDNA, resulting in plus-strand G→A mutations (8, 15, 17, 30, 33). In cells infected with wild-type HIV-1, Vif binds to hA3G and hA3F, forming a complex with a Cul5-based E3 ligase. The A3G proteins are then ubiquitinated and degraded by proteasomes (18, 31). Human APOBEC3B and human APOBEC3C are weakly active against HIV-1 but are potent inhibitors of SIV (2, 29). Other elements targeted by APOBEC3 family members include hepatitis B, endogenous murine long terminal repeat and non-long terminal repeat retroelements, and yeast retrotransposons (4–6, 26).

The interaction of Vif with hA3G is species specific (17). HIV-1 Vif binds to hA3G but not to rhesus macaque APOBEC3G (rhA3G) or AGM APOBEC3G (agmA3G). Conversely, SIV of AGMs (SIVagm) Vif binds to agmA3G but not to hA3G. SIVmac Vif is more permissive, binding to rhA3G, agmA3G, and hA3G. The determinant on hA3G that controls the species specificity of its interaction with Vif was mapped on a panel of hA3G-agmA3G chimeras. The specificity mapped to amino acid 128, which is Lys in hA3G but Asp in agmA3G (3, 16, 21, 28). Exchange of the Lys and Asp residues reverses the species specificity for binding to Vif. SIVagm Vif binds to D128K mutant hA3G but not to K128D mutant agmA3G. Conversely, HIV-1 Vif binds to K128D mutant agmA3G but not to D128K mutant hA3G. Modeling on the *Escherichia coli* cytidine deaminase predicts that amino acid 128 lies on an exposed loop that is positioned away from the enzyme's active site. The exchange does not affect the catalytic activity of the enzyme (21).

The objective of this study was to generate a genetically altered HIV-1 that could functionally interact with nonhuman

* Corresponding author. Mailing address: Infectious Disease Laboratory, The Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd., La Jolla, CA 92037. Phone: (858) 453-4100. Fax: (858) 554-0341. E-mail: Landau@salk.edu.

† Supplemental material for this article may be found at http://jvi.asm.org/.

primate APOBEC3 to counteract its antiviral activity. The approach taken was to identify amino acids in HIV-1 Vif that contribute to it species specificity and to alter these to resemble the sequence in SIV Vif. We found that a four-amino-acid sequence near the amino terminus of Vif which differs in charge between HIV-1 and SIV is important in determining the species specificity of the interaction with A3G. Alteration of the sequence resulted in a Vif that was able to counteract rhA3G and hA3G and allowed productive replication in human cells that stably expressed the deaminases. The mutation also allowed interaction with D128K mutant hA3G. These findings provide an important step toward the development of a primate model for HIV-1 replication.

MATERIALS AND METHODS

Vif expression vectors. A codon-optimized HIV-1 *vif* gene was assembled from synthetic oligonucleotides and amplified with a forward primer containing an EcoRI site and a reverse primer encoding a V5 epitope tag and an XhoI site (see Fig. S1 in the supplemental material). The PCR product was cloned into pcDNA3.1 at the EcoRI and XhoI sites to generate pcVif-Co. Mutations in Vif were generated in pcVif-Co by overlapping PCR and confirmed by nucleotide sequencing. Expression vector pcVifmac was constructed by amplification of the Vif open reading frame (ORF) from SIVmac239 with primers containing EcoRI and XhoI sites and encoding a carboxy-terminal V5 epitope tag. The PCR product was cleaved and cloned into pcDNA3.1 at the EcoRI and XhoI sites.

Viral plasmids. Single-cycle luciferase reporter viruses pNL-Luc-E$^-$R$^-$, pNL-Luc-E$^-$R$^-$Δ*vif*, and pSIVmac-Luc-E$^-$R$^-$ and replication-competent viruses NL-R$^-$ and NL-R$^-$V$^-$ have been previously described (17). The SEMQ mutation was introduced into pNL-Luc-E$^-$R$^-$ and NL43-R$^-$ by overlapping PCR in the fragment spanning the region between sites AgeI and PflmI (nucleotides 3485 to 5303) (data not shown). pNL4-3(Vif$_{mac}$) is a Δ*vif* NL4-3 Vpr$^-$ plasmid into which the SIVmac239 Vif ORF has been inserted in the *nef* gene. The plasmid was constructed by amplifying the SIVmac239 Vif ORF with primers containing NotI and XhoI restriction sites for cloning into *nef* at the same position as in NL-Luc.

Luciferase reporter virus assay. Vif function was analyzed as previously described (17, 21). Briefly, vesicular stomatitis virus G (VSV-G)-pseudotyped luciferase reporter viruses were produced in 293T cells by cotransfection of 2 μg of pNL-Luc-E$^-$R$^-$Δ*vif*, 1 μg of pcVif-Co.V5, 0.5 μg of an hA3G or rhA3G expression vector, and 0.5 μg of pcVSV-G. Virus was harvested 48 h posttransfection, and 1 ng of p24 was used to infect HOS.T4 cells in triplicate. Three days later, intracellular luciferase activity was measured with Luc-Lite Plus reagent (Packard) in a Topcount luminometer (Perkin-Elmer). The data are presented as mean counts per second ± the standard deviation. Vif expression in transfected cells was verified by immunoblot assay and probing with anti-V5 monoclonal antibody (MAb; Invitrogen). For titration experiments, 2 μg of pNL-Luc-E$^-$R$^-$, pNL-Luc-E$^-$R$^-$Δ*vif*, pNL-Luc-E$^-$R$^-$ Vif SEMQ, or pSIVmac-Luc-E$^-$R$^-$ was cotransfected with indicated amounts of rhA3F, hA3G, rhA3G, or D128K mutant hA3G and pcVSV-G. Viruses were normalized for p24 or p27, and 1 ng was used to measure their infectivity as described above.

Encapsidation of A3G. Virions were produced by cotransfection of 293T cells with 2 μg of pNL-Luc-E$^-$R$^-$, pNL-Luc-E$^-$R$^-$Δ*vif*, pNL-Luc-E$^-$R$^-$ Vif SEMQ with or without 1 μg of pcSIVmac Vif.V5, and 1.0 μg of hA3G.HA (where HA is hemagglutinin), rhA3G.HA, or D128K mutant hA3G.HA. The cells and supernatant were harvested 2 days posttransfection, and virus was concentrated by ultracentrifugation through 20% sucrose at 40,000 rpm for 1 h. A3G was detected in 10 ng of p24 and 20 μg of cell lysates by an immunoblot assay and probing with an anti-HA MAb. The blots were stripped and reprobed with an anti-Vif MAb, an anti-V5 MAb, or AIDS patient serum. The blots were also probed with an antitubulin MAb to control for equivalent loading.

Virus replication kinetics. The kinetics of viral replication was determined with HOS.T4.X4 cells that stably expressed hA3G.HA or rhA3G.HA. HOS.T4.X4 cells were infected with a VSV-G-pseudotyped pBABE-neo retroviral vector, and cells were selected with 0.5 mg/ml G418. Resistant clones were isolated and expanded, and rhA3G expression was confirmed by Western blot analysis. Cell clones were infected with 10 ng of p24 from wild-type, Δ*vif*, or Vif SEMQ replication-competent virus, and virus growth was measured over 15 days by p24 enzyme-linked immunosorbent assay.

RESULTS

Alteration of a three-amino-acid sequence in HIV-1 Vif allows interaction with rhA3G. A functional interaction of HIV-1 Vif with hA3G requires amino acid 128 of hA3G to be negative or neutral. Conversely, interaction of SIVagm Vif with agmA3G requires a positive charge at amino acid 128. We hypothesized that these charge requirements may result from the interaction of complementary charged amino acids in Vif and A3G. D128 in hA3G could interact with a positive charge in HIV-1 Vif, and K128 in agmA3G could interact with a negative charge in SIV Vif. To search for potential complementary amino acids in Vif that might interact with A3G amino acid 128, we searched known viral sequences to identify amino acid residues of Vif that were positively charged in HIV-1 and negative in SIV. In the SIVagm Vif sequences of the Los Alamos National Laboratory sequence database, there are seven conserved, negatively charged positions (E4, E17, E102, E104, D109, D/E122, and E215). Only two of the corresponding amino acids in HIV-1 Vif are positively charged (R4 and R15, corresponding to SIVagm Vif E4 and E17, respectively). HIV-1 Vif R15 is flanked by a nearby positive charge at R17, and these amino acids are conserved in HIV-1 Vif (R15 in >99% of isolates, and position 17 is either R or K in all sequenced viruses [82% R and 18% K]). We therefore focused on amino acids 14 to 17, which in HIV-1 Vif are DRMR. In SIVagm, positions 15 and 17 of Vif are neutral or negatively charged (Fig. 1A).

To test the role of amino acids 4 and 14 to 17 of HIV-1 Vif in determining the species specificity of the interaction with A3G, mutant Vif expression vectors were generated and their function was tested in a single-cycle infection assay. Single-cycle viruses were generated by cotransfection of 293T cells with a Δ*vif* HIV-1 luciferase reporter virus plasmid and a mutant or wild-type pcVif-HA expression vector. The pcVif plasmids expressed a Vif ORF that was codon optimized over its entire length to increase expression in the triple transfection (data not shown). A VSV-G expression vector was included in the transfection to generate infectious pseudotyped virus. The results of this analysis showed that a swap that included the R4 sequence (MEEEKR) maintained function against hA3G but did not counteract rhA3G or agmA3G. In contrast, the swap of the four-amino-acid sequence DRMR at positions 14 to 17 in the **SERQ** mutant (changes are in bold) was active against hA3G and rhA3G and partially active against agmA3G (Fig. 1B). Further analysis within positions 14 to 17 showed that changing only three of the four amino acids in the **SEMQ** mutant allowed interaction of HIV-1 Vif with rhA3G but not with agmA3G. Mutation of R14E and R17E to D**EME** was not sufficient to alter the interaction with A3G. Insertion of an E at position 17 in DRM**E**R was also insufficient. Immunoblot analysis of the cell lysates showed that the mutant proteins were expressed comparably to wild-type HIV-1 Vif (Fig. 1B, bottom). These findings suggest that positions 14 to 17 of HIV-1 Vif can influence the species specificity of the interaction with hA3G.

The role of this region in the interaction with rhA3G was tested with additional HIV-1 Vif mutants with changes at positions 14 to 17. Point mutations at each of the three positions (D14S, R15E, and R17Q) were tested (Fig. 2A). R15E failed

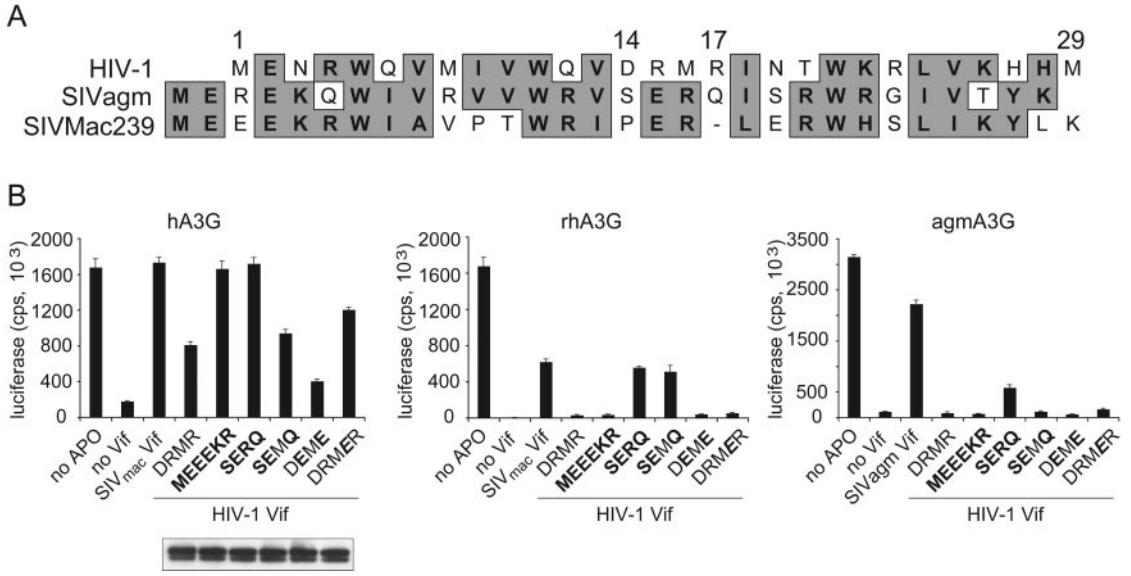

FIG. 1. A change of three amino acids in HIV-1 Vif allows functional interaction with rhA3G. (A) Alignment of HIV-1, SIVagm, and SIVmac Vif sequences. (B) Vif function of the indicated constructs was determined in a luciferase reporter virus assay by cotransfection of a Δ*vif* mutant HIV-1 luciferase reporter together with the respective Vif construct, empty vector (no APO), hA3G (left part), rhA3G (middle part), or agmA3G (right part) and pcVSV-G. The infectivity of the viruses was determined by infection of HOS.T4 cells with 1 ng of p24. The data are the averages of triplicates with the indicated standard deviations. cps, counts per second.

to function on hA3G or rhA3G, while D14S and R15E were active against hA3G but not against rhA3G. Thus, single mutations were not sufficient to change the species specificity of the interaction. **SE**MR, D**EMQ**, and **SRMQ** double mutants were also tested (Fig. 2B). All double mutants remained at least in part active against hA3G. Only D**EMQ** was partially active on rhA3G. Immunoblot analysis confirmed the equivalent expression of each of the mutant Vif proteins (Fig. 2A and

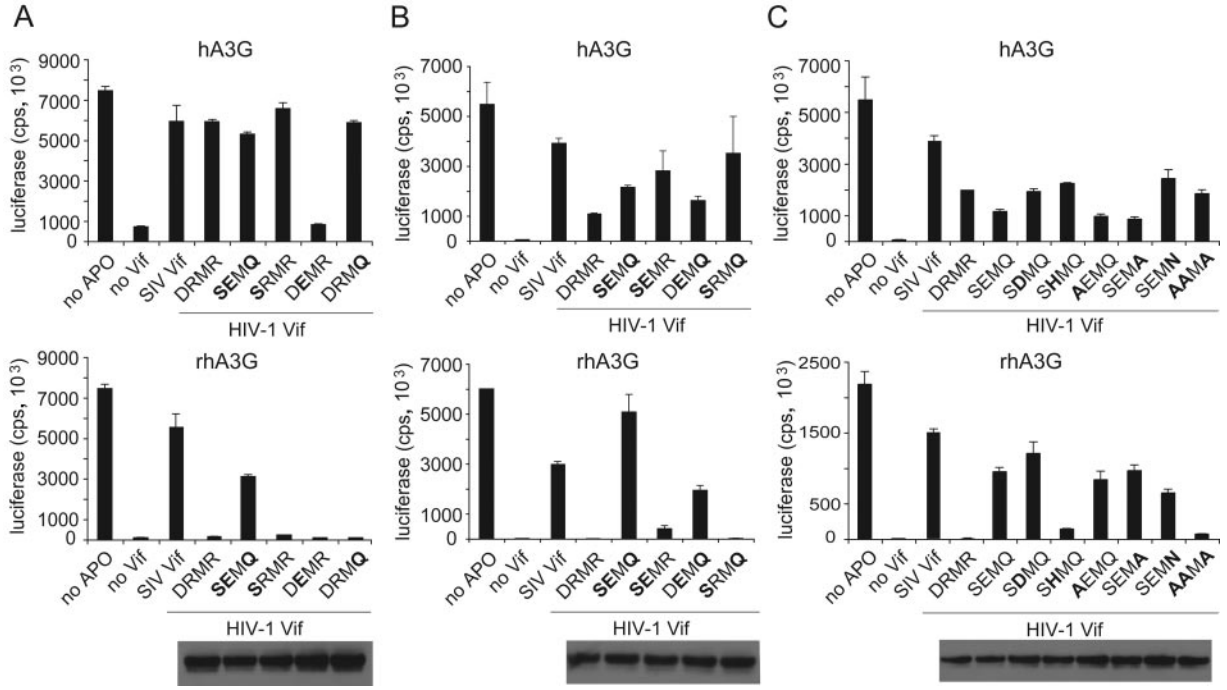

FIG. 2. The charge of the amino acids in SEMQ determines the ability to interact with rhA3G. (A, B) The effects of single (A) and double (B) mutations in the HIV-1 Vif DRMR sequence were tested against hA3G (top) and rhA3G (bottom) as described in the legend to Fig. 1. (C) The functional interaction of mutant HIV-1 Vif proteins containing charge changes with hA3G (top) and rhA3G was tested on Δ*vif* mutant HIV-1 luciferase reporters. The data are representative of three independent repetitions. cps, counts per second.

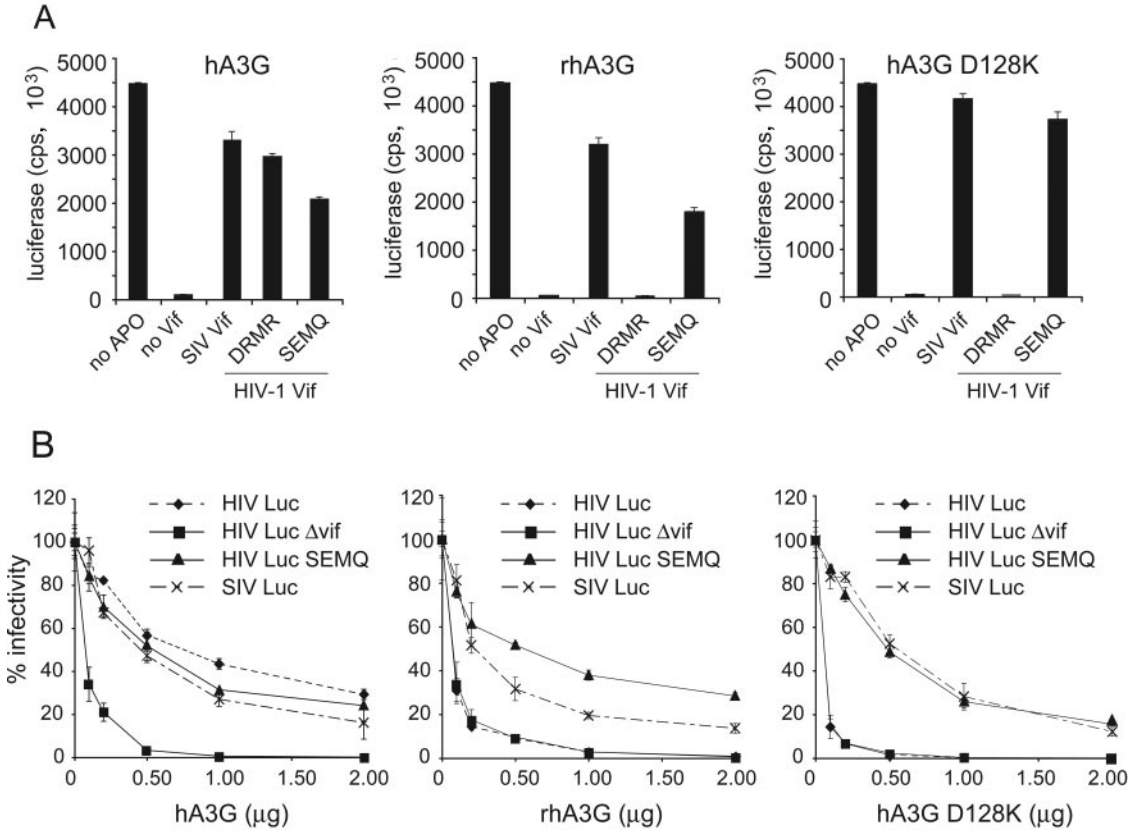

FIG. 3. SEMQ mutant Vif in *cis* is active against hA3G, rhA3G, and D128K hA3G. (A) The infectivity of Δ*vif* mutant HIV-1 Luc, wild-type HIV-1 Luc, SEMQ mutant HIV-1 Vif Luc, and Δ*vif* mutant HIV-1 Luc cotransfected with SIVmac Vif was tested against hA3G (left), rhA3G (middle), and D128K mutant hA3G (right). (B) Infectivity of luciferase reporter viruses that encode mutant Vif. Wild-type and Δ*vif* and SEMQ mutant luciferase HIV-1 and SIVmac reporters were produced in cells cotransfected with increasing amounts of hA3G (left), rhA3G (middle), or D128K hA3G (right). The infectivity of the virus produced in the absence of A3G was set to 100%, and the infectivity of mock-transfected cell supernatant (4×10^6 to 6×10^6 and 200 to 2,000 cps [counts per second], respectively) was set to 0%.

B, bottom). Taken together, these findings mapped the minimal required change to amino acids 14, 15, and 17. R15 and R17 appear to be the most important.

The charge at positions 14 to 17 is a determinant of the species specificity of the interaction with hA3G. To further probe the role of positions 14 to 17, we tested the dependence of charge on Vif function. The S**D**MQ and S**H**MQ mutants tested the requirement for a negative charge at position 15. The **A**EMQ mutant tested the importance of amino acid 14; the SEM**A** and SEM**N** mutants tested position 17; and the **AA**M**A** mutant tested positions 14, 15, and 17. All of the mutants interacted at least partially with hA3G (Fig. 2C, upper part). S**D**MQ but not S**H**MQ interacted with rhA3G, suggesting a requirement for a negative charge at position 15 (Fig. 2C, lower part). **A**EMQ, SEM**A**, and SEM**N** also interacted with rhA3G, suggesting that the identity of amino acids 14 and 17 is not critical but that they cannot be negative. **AA**M**A** was inactive against rhA3G, further demonstrating the importance of a negative charge at position 15. Taken together, these results suggest that the charge at amino acids 14 to 17 influences the interaction with rhA3G. Amino acid 15 must be negative, and amino acid 17 must be neutral.

The SEMQ mutation allows interaction of HIV-1 Vif with hA3G mutated at amino acid 128. The requirement for positive charges at positions 14 and 17 in HIV-1 Vif suggested an interaction with a negative charge on hA3G. Because of its role in determining the interaction with Vif, amino acid 128 was considered a likely candidate interaction site on hA3G. To determine whether this might be the case, we tested whether SEMQ HIV-1 Vif would interact with D128K hA3G. As expected, DRMR did not. Interestingly, SEMQ interacted with D128K mutant hA3G (Fig. 3A, right part). These results are consistent with a direct interaction of hA3G D128 with DRMR in Vif. In this analysis, the Vif mutations were introduced directly into the HIV-1 reporter construct. Because of the overlap with *pol*, the SEMQ mutation introduced three amino acid changes in IN (see Fig. S1 in the supplemental material). However, these did not interfere with the production of infectious reporter virus. In addition, the analysis showed that the Vif mutations were functional as expressed in *cis* in the context of the virus, arguing that the previous results were not simply the result of overexpression from the codon-optimized Vif expression vector.

To determine the relative efficiency with which the SEMQ mutation altered the Vif phenotype, the reporter viruses were produced from 293T cells cotransfected with hA3G, rhA3G, and D128K mutant hA3G expression vectors over a range of plasmid ratios. Reporter viruses that expressed wild-type

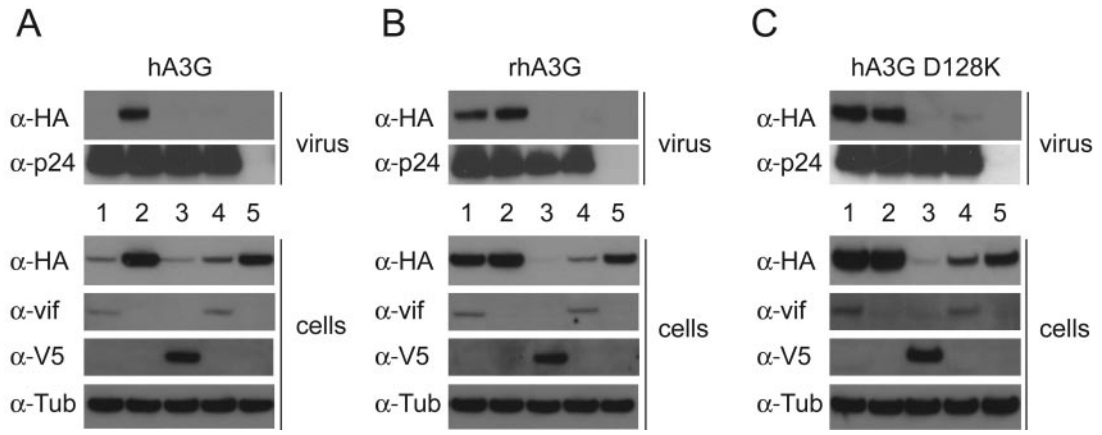

FIG. 4. SEMQ mutant Vif excludes hA3G, rhA3G, and D128K mutant hA3G from virions. (A) Encapsidation of hA3G (left), rhA3G (middle), and D128K mutant hA3G (right) was tested in wild-type HIV-1 (lane 1), Δ*vif* mutant HIV-1 (lane 2), Δ*vif* mutant HIV-1 plus SIVmac Vif (lane 3), SEMQ mutant Vif HIV-1 (lane 4), and a no-virus control. Virus produced in cells cotransfected with A3G was pelleted by ultracentrifugation. The viruses (top parts) and cell lysates (bottom parts) were analyzed by immunoblot assay and probing with an anti-HA MAb for A3G. Equal loading of virions was confirmed by probing with AIDS patient serum. Expression of HIV-1 Vif was confirmed in the cell lysates by probing the blot with an anti-HIV-1 Vif MAb, and that of SIVmac Vif was confirmed by probing the blot with an anti-V5 MAb. The blot was probed with an antitubulin MAb to confirm equal loading.

HIV-1 Vif, SEMQ Vif, and SIVmac Vif were all similarly resistant to hA3G and differed from Δ*vif* mutant virus, which was sensitive (Fig. 3B, left part). A reporter virus that expressed HIV-1 Vif was inhibited by rhA3G (Fig. 3B, middle part), in contrast to viruses that expressed SEMQ Vif and SIVmac Vif, which were resistant. Viruses that expressed HIV-1 Vif, SEMQ mutant HIV-1 Vif, and SIVmac Vif were all similarly resistant to D128K mutant hA3G (Fig. 3B, right part). Taken together, the results show that the SEMQ mutation allows HIV-1 Vif to counteract rhA3G and D128K mutant hA3G with an efficiency comparable to that of SIVmac Vif. These results show that the SEMQ gene is functional as expressed by the virus and that the alteration to IN was not deleterious.

SEMQ mutant Vif reduces A3G virion encapsidation. To biochemically assess SEMQ mutant Vif function, its effect on A3G virion encapsidation and steady-state intracellular level was measured. 293T cells were transfected with Δ*vif* mutant HIV-1, wild-type HIV-1, or Δ*vif* mutant HIV-1 plus pcSIVmac-vif or SEMQ mutant HIV-1 Vif and an A3G expression vector. The virions were pelleted, and their A3G content was quantitated by immunoblot analysis. hA3G was present in Δ*vif* virions but not in controls that lacked viral DNA or that expressed SIVmac Vif or SEMQ mutant Vif (Fig. 4A, upper part). The cell lysates showed that SIVmac Vif and SEMQ mutant Vif reduced the steady-state level of hA3G (Fig. 4A, lower part). HIV-1 Vif and SIVmac Vif were detected with anti-Vif and anti-V5 epitope tag MAbs, respectively. SIVmac Vif appeared to be present at higher levels, probably because it was supplemented in *trans*. HIV-1 Vif did not exclude rhA3G from virions (Fig. 4B, upper part). In contrast, SIVmac Vif and SEMQ mutant Vif effectively prevented the encapsidation of rhA3G. SIVmac Vif and SEMQ mutant Vif correspondingly reduced steady-state rhA3G levels in the cell lysates (Fig. 4B, lower part). Similarly, HIV-1 Vif did not exclude D128K mutant hA3G from virions but SIVmac and SEMQ mutant Vif were effective and reduced the steady-state levels of D128K mutant hA3G in the cell lysates (Fig. 4B, upper and lower parts). SEMQ mutant Vif was expressed at amounts comparable to wild-type Vif in each panel, suggesting that the effect was not caused by differences in levels of Vif.

HIV-1 with the SEMQ mutation replicates in human cells that stably express rhA3G. To test whether introduction of the SEMQ mutation would allow HIV-1 to productively replicate in cells that express rhA3G, the mutation was introduced into replication-competent NL4-3. The SEMQ mutant NL4-3 virus, along with control Δ*vif* mutant and wild-type NL4-3, was used to infect HOS.T4.X4 cells that stably expressed CD4/CXCR4 and hA3G or rhA3G or that lacked A3G. Clones of HOS cells that expressed moderate (clone 8) or high (clone 5) levels of rhA3G were used (Fig. 5A). Virus replication kinetics was measured over 2 weeks. The three viruses replicated similarly in cells that lacked A3G (Fig. 5B). On cells that express hA3G, the wild-type and SEMQ mutant viruses replicated but the Δ*vif* mutant virus replicated poorly (Fig. 5C). On clone 8 and clone 5 rhA3G cells, the SEMQ mutant virus replicated efficiently, unlike the Δ*vif* mutant and wild-type viruses, which failed to replicate (Fig. 5D). These results show that the SEMQ mutation allows efficient replication of HIV-1 on cells that expressed relatively high levels of rhA3G.

rhA3F does not block HIV-1 replication. hA3F has previously been shown to block Δ*vif* mutant HIV-1 infectivity in single-round reporter virus assays (14, 27, 34). To test whether A3F restricts HIV-1 in a species-specific manner similar to that of A3G, we cloned rhA3F (see Fig. S2 in the supplemental material) and tested its activity against wild-type, Δ*vif* mutant, and SEMQ mutant HIV-1 reporter viruses. As expected, hA3F inhibited Δ*vif* mutant but not wild-type HIV-1 (14, 27, 34). In contrast, hA3F inhibited the SEMQ mutant virus. This suggested that the SEMQ mutation caused Vif to lose its ability to counteract hA3F (Fig. 6A). rhA3F inhibited wild-type HIV-1 and the SEMQ mutant. hA3F and rhA3F inhibited Δ*vif* mutant SIVmac, and this was partially overcome by SIVmac Vif, consistent with a recent report by Zennou and Bieniasz (32) which

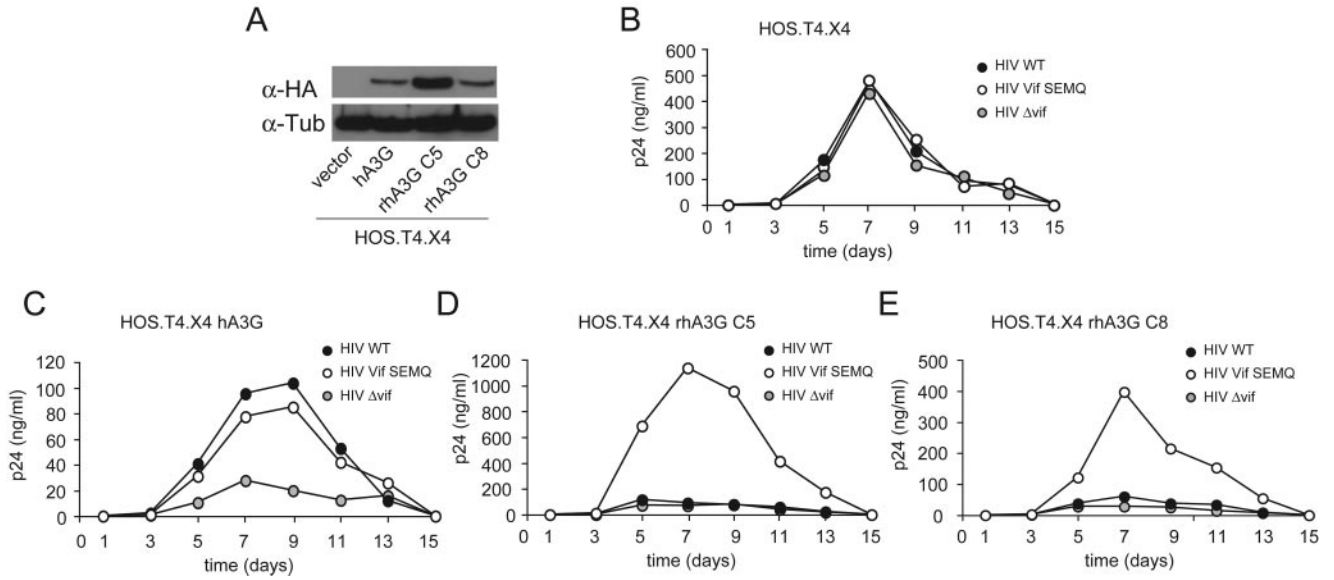

FIG. 5. Viral replication kinetics in cells expressing hA3G or rhA3G. (A) hA3G and rhA3G stably expressed by HOS.T4.X4 cells derived by retroviral vector transduction were detected by an immunoblot assay and probing with an anti-HA MAb. The parental cells lack A3G. (B to E) Replication kinetics of wild-type (WT), Δ*vif* mutant, or SEMQ mutant Vif HIV-1 in HOS.T4.X4 cells expressing no A3G, hA3G, or different amounts of rhA3G were determined. Supernatant capsid p24 was quantitated over 15 days.

showed that SIVmac Vif was partially active against rhA3F. Our data suggested that the interaction between Vif and A3F is species specific and that SEMQ mutant Vif fails to interact with rhA3F.

To determine whether rhA3F blocks the replication of HIV-1, we infected HOS cells that stably express CD4/CXCR4 and rhA3F with replication-competent wild-type, Δ*vif* mutant, or SEMQ mutant NL4-3 or NL4-3(Vif_{mac}), an HIV-1 strain that contains an engineered SIV_{mac} *vif* gene in the *nef* position. The HOS cells expressed moderate (C1) or high (C4) levels of rhA3F (Fig. 6B). All viruses replicated well in the parental HOS.CD4.CXCR4 cell line (Fig. 6B). Surprisingly, intermediate and high levels of rhA3F had no effect on the replication kinetics. Some differences in peak p24 production were evident among the HOS cell clones, but these were caused by differences in coreceptor expression (data not shown). Taken together, these data demonstrate that although rhA3F is active against HIV-1 in the single-cycle reporter virus assay, it does not block virus replication under more physiological conditions.

DISCUSSION

We report here progress in addressing the APOBEC3 block to HIV-1 replication in nonhuman primate cells. Changing the sequence DRMR at positions 14 to 17 to SEMQ, which resembles the SIV sequence, allowed it to counteract the antiviral activity of rhA3G and agmA3G. SEMQ mutant Vif induced rhA3G degradation and prevented its encapsidation. The SEMQ mutation allowed the production of infectious reporter virus in cells cotransfected with rhA3G, and in replication-competent HIV-1, it allowed productive replication in human cells that stably expressed relatively high levels of hA3G or rhA3G. The mutant Vif protein was inactive against rhA3F in reporter virus assays, but HIV-1 was able to productively replicate in cells that stably expressed rhA3F. The DRMR sequence is highly conserved in HIV Vif, consistent with a role as an interaction site for A3G. D14, R15, and M16 are 99% conserved in database HIV-1 sequences, while R17 is conserved in 82% of isolates and replaced with Lys in 18% of sequences.

The DRMR sequence in HIV-1 Vif is important for interaction with APOBEC3G but is not the only region that is required for a functional interaction. The SEMQ mutant gained the ability to interact with rhA3G but did not lose its ability to interact with hA3G. This suggests that the interaction of Vif with APOBEC3G is stabilized by additional binding sites. With the exception of Vif R15E, which lost its function against hA3G, mutations in this region in general had little effect on the interaction with hA3G. Thus, the DRMR region is critical for the strength of binding with A3G but is not the sole determinant of its interaction with hA3G. Consistent with this finding, HIV-1 Vif binds with low affinity to D128K hA3G in coimmunoprecipitation experiments (21). This interaction was not sufficient to counteract the antiviral function.

A prediction of this work is that position 128 of A3G interacts with Vif at amino acid 15 or 17. This is suggested by the finding that replacement of R15 and R17 in HIV-1 Vif with neutral or negatively charged residues allowed interaction with rhA3G, which is positively charged at position 128. In addition, mutations at R15 and R17 allowed HIV-1 Vif to interact with D128K mutant hA3G, which contains only a single amino acid difference from wild-type hA3G. The complementarity of the changes in Vif and APOBEC3G suggests a direct electrostatic interaction at these positions, although indirect effects on the conformation of the proteins cannot be ruled out.

An earlier report by Kar et al. (13) described findings that

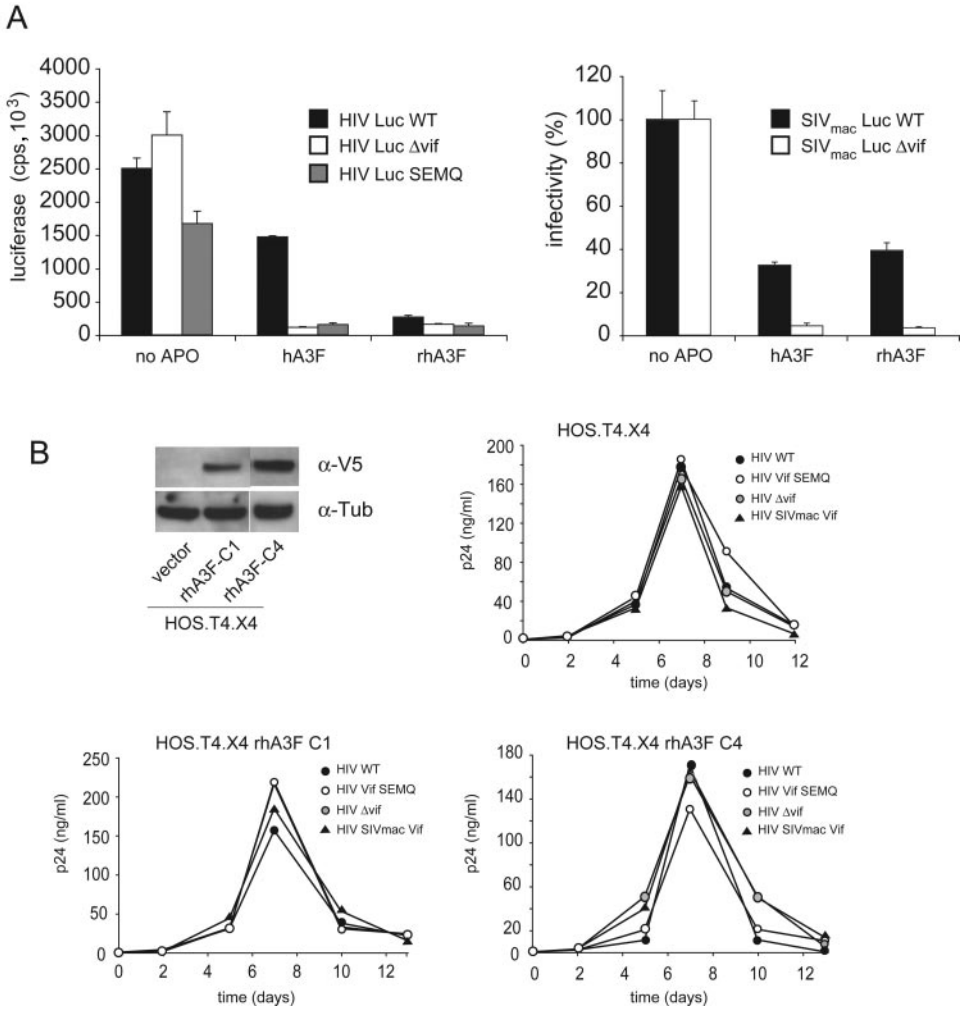

FIG. 6. rhA3F does not block HIV-1 replication. (A) The antiviral effects of hA3F and rhA3F were tested in a single-cycle luciferase reporter virus assay. Virus was produced by cotransfection of wild-type, Δ*vif* mutant, or SEMQ mutant NL-Luc or wild-type (WT) or Δ*vif* mutant SIVmac Luc and the indicated A3F expression plasmid. HOS.T4 cells were infected with 1 ng of reporter virus, and intracellular luciferase activity was measured 3 days postinfection. (B) Immunoblot analysis of V5-tagged rhA3F expression in HOS.T4.X4 cell clones or control cell clones (left). Replication kinetics of wild-type, Δ*vif* mutant, or SEMQ mutant NL4-3(Vif_{mac}) and NL4-3(Vif_{mac}), which expresses SIVmac239 Vif in the *nef* position of NL4-3 on control and rhA3F-expressing HOS.T4.X4 cell clones. The cells expressed intermediate (C1) or high (C4) levels of rhA3F. Supernatant p24 was measured over 2 weeks. cps, counts per second.

appear to be at odds with those presented here. In that work, an HIV-1 *vif* gene was inserted into the *nef* position of Δ*vif* mutant SIVmac239. The chimeric virus replicated in rhesus T cells, suggesting that HIV Vif-1 can substitute for Vif in SIVmac239 and implying that HIV-1 Vif functionally interacts with rhA3G. In contrast, we found that low levels of rhA3G blocked HIV-1 replication. The explanation for this difference is not obvious but could be related to the expression of Vif in the *nef* position instead of its native context or could be related to the use of primary rhesus T cells, which may express relatively low levels of rhA3G.

SEMQ mutant Vif did not interact with rhA3F, probably because the binding site on Vif for A3F is not identical to that for A3G (24). This raised the question of whether rhA3F is an important restriction factor for HIV-1 in nonhuman primate cells. In humans, hA3F is expressed in T cells and is active against HIV-1 (14, 27, 34). rhA3F is likely to be similarly expressed in nonhuman primates. In single-cycle luciferase virus analyses, rhA3F inhibited HIV-1 and the inhibition was not reversed by Vif. SIVmac was also inhibited by rhA3F, but this was somewhat reversed by SIV Vif. In contrast, our analysis of replication-competent virus on cells that stably expressed rhA3F led to somewhat different conclusions. In this analysis, which more closely mimics physiological conditions, rhA3F did not inhibit HIV-1 replication. This suggests that rhA3F is not an impediment to HIV-1 replication in nonhuman primate cells. The SEMQ mutation in Vif may be sufficient to overcome the APOBEC3 barrier to HIV-1 replication in nonhuman primates without the need for additional mutational alteration.

Trim5α remains as a barrier to HIV-1 replication in nonhuman primate cells. As a result, the SEMQ mutant does not replicate in primary rhesus cells (data not shown). Owens et al. (20) showed that various mutations in the HIV-1 capsid, most

in the vicinity of the cyclophilin binding loop, partially allowed the virus to escape rhTrim5α. The combination of these mutations could be helpful in the establishment of a novel primate model for AIDS.

ACKNOWLEDGMENTS

We thank Hui Chen and Qin Yu for critical reading of the manuscript.

This work was funded by grants from the National Institutes of Health (AI51686 and DA14494) and the American Foundation for AIDS Research. N.R.L. is an Elizabeth Glaser Fellow of the Pediatric AIDS Foundation.

REFERENCES

1. **Besnier, C., Y. Takeuchi, and G. Towers.** 2002. Restriction of lentivirus in monkeys. Proc. Natl. Acad. Sci. USA **99:**11920–11925.
2. **Bishop, K. N., R. K. Holmes, A. M. Sheehy, N. O. Davidson, S. J. Cho, and M. H. Malim.** 2004. Cytidine deamination of retroviral DNA by diverse APOBEC proteins. Curr. Biol. **14:**1392–1396.
3. **Bogerd, H. P., B. P. Doehle, H. L. Wiegand, and B. R. Cullen.** 2004. A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. Proc. Natl. Acad. Sci. USA **101:**3770–3774.
4. **Bogerd, H. P., H. L. Wiegand, B. P. Doehle, K. K. Lueders, and B. R. Cullen.** 2006. APOBEC3A and APOBEC3B are potent inhibitors of LTR-retrotransposon function in human cells. Nucleic Acids Res. **34:**89–95.
5. **Chen, H., C. E. Lilley, Q. Yu, D. V. Lee, J. Chou, I. Narvaiza, N. R. Landau, and M. D. Weitzman.** 2006. APOBEC3A is a potent inhibitor of adeno-associated virus and retrotransposons. Curr. Biol. **16:**480–485.
6. **Esnault, C., O. Heidmann, F. Delebecque, M. Dewannieux, D. Ribet, A. J. Hance, T. Heidmann, and O. Schwartz.** 2005. APOBEC3G cytidine deaminase inhibits retrotransposition of endogenous retroviruses. Nature **433:**430–433.
7. **Etemad-Moghadam, B., D. Rhone, T. Steenbeke, Y. Sun, J. Manola, R. Gelman, J. W. Fanton, P. Racz, K. Tenner-Racz, M. K. Axthelm, N. L. Letvin, and J. Sodroski.** 2002. Understanding the basis of $CD4^+$ T-cell depletion in macaques infected by a simian-human immunodeficiency virus. Vaccine **20:**1934–1937.
8. **Harris, R. S., K. N. Bishop, A. M. Sheehy, H. M. Craig, S. K. Petersen-Mahrt, I. N. Watt, M. S. Neuberger, and M. H. Malim.** 2003. DNA deamination mediates innate immunity to retroviral infection. Cell **113:**803–809.
9. **Harris, R. S., and M. T. Liddament.** 2004. Retroviral restriction by APOBEC proteins. Nat. Rev. Immunol. **4:**868–877.
10. **Hatziioannou, T., S. Cowan, S. P. Goff, P. D. Bieniasz, and G. J. Towers.** 2003. Restriction of multiple divergent retroviruses by Lv1 and Ref1. EMBO J. **22:**385–394.
11. **Himathongkham, S., and P. A. Luciw.** 1996. Restriction of HIV-1 (subtype B) replication at the entry step in rhesus macaque cells. Virology **219:**485–488.
12. **Hofmann, W., D. Schubert, J. LaBonte, L. Munson, S. Gibson, J. Scammell, P. Ferrigno, and J. Sodroski.** 1999. Species-specific, postentry barriers to primate immunodeficiency virus infection. J. Virol. **73:**10020–10028.
13. **Kar, S., P. Cummings, and L. Alexander.** 2003. Human immunodeficiency virus type 1 Vif supports efficient primate lentivirus replication in rhesus monkey cells. J. Gen. Virol. **84:**3227–3231.
14. **Liddament, M. T., W. L. Brown, A. J. Schumacher, and R. S. Harris.** 2004. APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 in vivo. Curr. Biol. **14:**1385–1391.
15. **Mangeat, B., P. Turelli, G. Caron, M. Friedli, L. Perrin, and D. Trono.** 2003. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature **424:**99–103.
16. **Mangeat, B., P. Turelli, S. Liao, and D. Trono.** 2004. A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. J. Biol. Chem. **279:**14481–14483.
17. **Mariani, R., D. Chen, B. Schröfelbauer, F. Navarro, R. Konig, B. Bollman, C. Munk, H. Nymark-McMahon, and N. R. Landau.** 2003. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell **114:**21–31.
18. **Mehle, A., B. Strack, P. Ancuta, C. Zhang, M. McPike, and D. Gabuzda.** 2004. Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. J. Biol. Chem. **279:**7792–7798.
19. **Munk, C., S. M. Brandt, G. Lucero, and N. R. Landau.** 2002. A dominant block to HIV-1 replication at reverse transcription in simian cells. Proc. Natl. Acad. Sci. USA **99:**13843–13848.
20. **Owens, C. M., B. Song, M. J. Perron, P. C. Yang, M. Stremlau, and J. Sodroski.** 2004. Binding and susceptibility to postentry restriction factors in monkey cells are specified by distinct regions of the human immunodeficiency virus type 1 capsid. J. Virol. **78:**5423–5437.
21. **Schröfelbauer, B., D. Chen, and N. R. Landau.** 2004. A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). Proc. Natl. Acad. Sci. USA **101:**3927–3932.
22. **Sheehy, A. M., N. C. Gaddis, J. D. Choi, and M. H. Malim.** 2002. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature **418:**646–650.
23. **Shibata, R., H. Sakai, M. Kawamura, K. Tokunaga, and A. Adachi.** 1995. Early replication block of human immunodeficiency virus type 1 in monkey cells. J. Gen. Virol. **76**(Pt. 11)**:**2723–2730.
24. **Simon, V., V. Zennou, D. Murray, Y. Huang, D. D. Ho, and P. D. Bieniasz.** 2005. Natural variation in Vif: differential impact on APOBEC3G/3F and a potential role in HIV-1 diversification. PLoS Pathog. **1:**e6.
25. **Stremlau, M., C. M. Owens, M. J. Perron, M. Kiessling, P. Autissier, and J. Sodroski.** 2004. The cytoplasmic body component TRIM5α restricts HIV-1 infection in Old World monkeys. Nature **427:**848–853.
26. **Turelli, P., S. Vianin, and D. Trono.** 2004. The innate antiretroviral factor APOBEC3G does not affect human LINE-1 retrotransposition in a cell culture assay. J. Biol. Chem. **279:**43371–43373.
27. **Wiegand, H. L., B. P. Doehle, H. P. Bogerd, and B. R. Cullen.** 2004. A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. EMBO J. **23:**2451–2458.
28. **Xu, H., E. S. Svarovskaia, R. Barr, Y. Zhang, M. A. Khan, K. Strebel, and V. K. Pathak.** 2004. A single amino acid substitution in human APOBEC3G antiretroviral enzyme confers resistance to HIV-1 virion infectivity factor-induced depletion. Proc. Natl. Acad. Sci. USA **101:**5652–5657.
29. **Yu, Q., D. Chen, R. Konig, R. Mariani, D. Unutmaz, and N. R. Landau.** 2004. APOBEC3B and APOBEC3C are potent inhibitors of simian immunodeficiency virus replication. J. Biol. Chem. **279:**53379–53386.
30. **Yu, Q., R. Konig, S. Pillai, K. Chiles, M. Kearney, S. Palmer, D. Richman, J. M. Coffin, and N. R. Landau.** 2004. Single-strand specificity of APOBEC3G accounts for minus-strand deamination of the HIV genome. Nat. Struct. Mol. Biol. **11:**435–442.
31. **Yu, X., Y. Yu, B. Liu, K. Luo, W. Kong, P. Mao, and X. F. Yu.** 2003. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science **302:**1056–1060.
32. **Zennou, V., and P. D. Bieniasz.** 2006. Comparative analysis of the antiretroviral activity of APOBEC3G and APOBEC3F from primates. Virology [Online.] doi:10.1016/j.virol.2005.12.035.
33. **Zhang, H., B. Yang, R. J. Pomerantz, C. Zhang, S. C. Arunachalam, and L. Gao.** 2003. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. Nature **424:**94–98.
34. **Zheng, Y. H., D. Irwin, T. Kurosu, K. Tokunaga, T. Sata, and B. M. Peterlin.** 2004. Human APOBEC3F is another host factor that blocks human immunodeficiency virus type 1 replication. J. Virol. **78:**6073–6076.