The virus infectivity factor (Vif) is a protein encoded by most primate lentiviruses. Recent evidence suggests that HIV-1 Vif reduces the intracellular levels of the host cytidine deaminase APOBEC3G (Apo3G) and inhibits its packaging into virions. These functions of Vif are thought to be species-specific. Accordingly, HIV-1 Vif can target only human Apo3G (hApo3G), whereas, African green monkey (mangabeys) simian immunodeficiency virus (SIVagm) Vif can inhibit African green monkey but not human Apo3G. Consistent with this, we found that SIVagm Vif does not affect the stability of exogenously and endogenously expressed hApo3G and does not prevent packaging of exogenous and endogenous hApo3G into SIVagm virions. Nevertheless, SIVagm Vif supported spreading infection of SIVagm virus in the hApo3G-positive human A3.01 T cell line and rescued infectivity of viruses produced from Apo3G-expressing HeLa cells. Sequence analysis verified that SIVagm Vif inhibited the accumulation of hApo3G-induced mutations, suggesting that SIVagm Vif is indeed active in human cells. Our data suggest that SIVagm Vif can inhibit hApo3G activity without inducing its intracellular degradation or preventing its packaging into virions.

Replication of primate lentiviruses in their natural hosts is generally nonpathogenic; however, cross-species transmission of these viruses can result in highly pathogenic phenotypes. Indeed, there is evidence that HIV-1 and HIV, type 2, evolved from zoonotic transmission of SIV from chimpanzees and sooty mangabeys, respectively (1–3). Crossover of a virus into a new host poses a significant challenge and requires adaptation by the virus to its new environment. Human APOBEC3G (hApo3G) was recently identified as a host factor that inhibits replication of vif-defective HIV-1 (4). hApo3G is a host cytidine deaminase that in the absence of Vif is packaged into HIV virions and causes hypermutation of the viral genome. Expression of Vif reduces the intracellular expression level of hApo3G and inhibits packaging of the deaminase (5–14).

Previous studies reported that Vif from African green monkey simian immunodeficiency virus (SIVagm) does not function in human cells (15) and does not target hApo3G (16–18). Consequently, the function of SIVagm Vif is thought to be highly species-specific. This is explained by the ability of Vif to interact selectively with its respective Apo3G counterpart (12). Binding of HIV-1 Vif to hApo3G was shown to be necessary but not sufficient to overcome the inhibitory effects of the deaminase (11, 14). Moreover, co-expression of Vif was reported to induce ubiquitination of hApo3G leading to subsequent proteasome degradation (11, 13, 14, 19, 20). More recent studies demonstrate that the selective targeting of human and agmApo3G by HIV-1 and SIVagm Vif, respectively, is controlled by a single amino acid residue in Apo3G (16, 17, 21, 22). It remains to be shown, however, why mutation of residue 128 from aspartic acid to lysine in hApo3G renders the protein insensitive to HIV-1 Vif but now makes it sensitive to SIVagm Vif. Although this would be easily explained by a loss or gain, respectively, of Vif-Apo3G interactions (16, 17), at least one study did not observe a significant interference of the D128K mutation with binding to HIV-1 Vif (21). At any rate, the insensitivity of hApo3G to SIVagm Vif in the biochemical assays predicts that SIVagm is unable to establish a spreading infection in Apo3G-positive human cells. Yet recent evidence demonstrates that such species-specific restriction of Vif function may not be absolute. In fact, several groups have reported on the replication of SIVagm viruses in cultured human macrophages and PBMCs, which are generally known to be Apo3G-positive (23, 24).

The goal of this study was to investigate the role of Vif in the replication of SIVagm in human cells and thus to determine its possible role in the zoonotic transmission of SIVagm into humans. We found that SIVagm Vif is indeed active in human cells and promotes spreading infection of SIVagm in the Apo3G-positive human A3.01 T cell line by preventing the accumulation of Apo3G-induced hypermutations.

**EXPERIMENTAL PROCEDURES**

**Plasmids—**The full-length molecular clones pSIVagm9063 (25) and HIV-1NL4-3 (26) were used for the production of wild type infectious virus. A vif-defective variant of SIVagm9063 carrying a 29-bp deletion in the vif gene (nucleotide positions 108–137 from the start of the vif gene) was constructed by two-step PCR. The resulting construct expresses all viral proteins except for Vif. For transient expression of Vif, the subgenomic expression vector pNL-A1 was employed (27). For the expression of SIVagm Vif protein, the vif sequence of the SIVagm9063 isolate (GenBank™ accession number L40990) was PCR-amplified and inserted between the BssHII and EcoRI sites of pNL-A1, resulting in...
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pNL-A1/Agm.Vif. Plasmid pcDNA-Apo3G for the expression of human Apo3G has been described previously (5).

Antisera—Exogenously expressed Apo3G was identified by using a polyclonal antibody raised against recombinant human Apo3G (5). The serum reacts with exogenously expressed human and agmApo3G but does not recognize endogenous Apo3G. The antisera is publicly available through the National Institutes of Health AIDS Research and Reference Reagent Program. A polyclonal antibody to SIVagm Vif was prepared for immunizing rabbits with purified recombinant protein. A monoclonal antibody to Vif (monoclonal antibody 319) was used for all immunoblots analyses and was obtained from Michael Malm through the National Institutes of Health AIDS Research and Reference Reagent Program. SIVagm capsid (CA) proteins were identified by using a rabbit polyclonal antibody provided by Vanessa Hirsch (28). For detection of endogenous hApo3G, a peptide antiserum to APOBEC3G obtained from Warner Greene was used (10). A monoclonal antibody to α-tubulin (DM 1A) was obtained from Sigma.

Tissue Culture and Transfections—HeLa cells were propagated in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (FBS). LuSIV cells are derived from CEMx174 cells and contain a luciferase indicator gene under the control of the SIVmac239 LTR. These cells were obtained through the National Institutes of Health AIDS Research and Reference Reagent Program and were maintained in complete RPMI 1640 medium supplemented with 10% FBS. Two days prior to transfection, cells were grown to a density of 2 × 10^6 cells/ml. Electroporations were carried out using a GENE PULSER II (Bio-Rad). The electroporation parameters were 200 V and 975 microfarads.

Electroporation of A3.01 Cells—A3.01 cells were collected, washed once with Opti-MEM (Invitrogen), and resuspended in electroporation medium (RPMI 1640, 10 mM t-glucose, 0.1 mM dithiothreitol). A total of 20 μg DNA was mixed per 5 × 10^6 cells. Electroporations were carried out using a GENE PULSER II (Bio-Rad). The electroporation parameters were 200 V and 975 microfarads.

After electroporation, cells were transferred to complete RPMI 1640 medium supplemented with 10% FBS. Cells and virus-containing supernatants were collected 3 days later and analyzed by immunoblotting.

Immunoblotting—For immunoblot analysis of intracellular proteins, whole-cell lysates were prepared as follows. Cells were washed once with PBS, suspended in PBS (200 μl per 5 × 10^6 cells), and mixed with an equal volume of 2× sample buffer (4% SDS, 125 mM Tris-HCl, pH 6.8, 10% 2-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue). To analyze virus-associated proteins, cell-free filtered supernatants from transduced HeLa cells (7 ml) were pelleted (75 min, 35,000 rpm) through a 20% sucrose solution. Immediately prior to adding concentrated virus stocks (700 μl), the step gradients were overlaid with 100 μl of either PBS or 1% Triton X-100 in PBS. Samples were then centrifuged in a SW55Ti rotor for 60 min at 35,000 rpm at 4 °C. Three fractions (a–c) of 1.1 ml each were collected from the top and combined with 350 μl of 4× sample buffer (8% SDS, 250 mM Tris-HCl, pH 6.8, 20% 2-mercaptoethanol, 20% glycerol, and 0.005% bromophenol blue). Aliquots (120 μl) of each fraction of the step gradients were subsequently processed for immunoblotting.

DNA PCR—Proviral DNA was extracted from infected cells 14 days after infection by using the DNeasy Tissue kit (Qiagen Inc., Valencia, CA). LTR sequences were amplified by DNA PCR using the Expand Long Template PCR System (Roche Diagnostics) and primers SIVagm-LTR-5/(CCA GGG AGA TCA GTG TGG AAA C) and SIVagm-LTR-3/(GAG TTT CTC CCG CCT GGG TCA G). Sequences from the V3 region in SIVagm env were amplified using primers SIVagm-V3-5’/(CTG TAA TAG TTC TTT TTA ACA A) and SIVagm-V3-3’/(AAA CTG TTA CCG AGA TCA TATT TT). PCR products were inserted into pGEM-T vector (Promega Corp., Madison, WI). 15–18 individual clones encompassing a total of 3,700 to 4,772 nucleotides were then sequenced by the National Institutes of Health AIDS Research and Reference Reagent Program. SIVagm capsid (CA) proteins were identified by using a rabbit polyclonal antibody provided by Vanessa Hirsch (28). For detection of exogenous hApo3G, a peptide antiserum to APOBEC3G obtained from Warner Greene was used (10). A monoclonal antibody to α-tubulin (DM 1A) was obtained from Sigma.

RESULTS

Vif-dependent Replication of SIVagm in Human Cell Lines—Although it was reported previously that SIVagm Vif does not function in human cells (15) and does not target hApo3G in transiently transfected 293T cells (16–18), other reports observed SIVagm replication in human PBMC albeit in a donor-dependent manner (23, 24). Replication of SIVagm viruses in human cells may, of course, face multiple restrictions unrelated to Vif function; however, it is possible that donor-dependent variations in the Apo3G expression levels contribute to some extent to the ability or inability of SIVagm viruses to replicate in human and non-human primate cell types. We started out by comparing the expression of Apo3G in various tissues that include a number of human and non-human primate cell lines, as well as human macrophages and human PBMC derived from four independent donors (Fig. 1A). All samples were normalized for equal protein content prior to gel electrophoresis and were analyzed by immunoblotting by using an Apo3G-specific peptide antibody (10). As can be seen in Fig. 1A, Apo3G expression varied significantly among different cell types. Of note, even though all human PBMC tested positive for Apo3G, there was significant donor-dependent variation in the Apo3G expression levels.

To address the question of whether differences in the Apo3G expression level control the replication of wild type and vif-defective SIVagm in human cells, we chose as model systems A3.01 cells, which express Apo3G at levels comparable to those found in most PBMC or macrophages (see Fig. 1A). CEM-SS cells, which are commonly used for propagation of SIVagm viruses (30) and express only low levels of Apo3G (Fig. 1A), were analyzed in parallel. To address the role of Vif in the replication of SIVagm in human cells, we constructed a vif-defective variant of SIVagm9063 to compare its replication fitness in human cells to that of wild type virus. To inactivate the vif gene, PCR-based mutagenesis was used to introduce an out-of-frame deletion near the N terminus of Vif. Virus stocks of full-length and vif-defective SIVagm9063 were prepared in transfected HeLa cells and used for the infection of A3.01 or...
CEM-SS cells (Fig. 1B). Most interestingly, wild type SIVagm replicated in A3.01 cells albeit at significantly reduced levels when compared with CEM-SS cells (Fig. 1B), presumably due to the presence of relatively high levels of hApo3G in A3.01 cells (see Fig. 1A). In contrast, viif-defective SIVagm replicated only very inefficiently in A3.01 cells, providing the first evidence that SIVagm Vif may indeed be active against hApo3G.

In CEM-SS cells, both wild type and viif-defective SIVagm viruses replicated with similar efficiency despite the presence of low levels of hApo3G. The fact that replication of viif-defective SIVagm is not impaired in CEM-SS cells indicates that low levels of cellular hApo3G are not detrimental to virus replication even in the absence of Vif. This is true for both SIVagm and HIV-1 because CEM-SS cells are fully permissive for HIV-1 as well (31) (see supplemental Fig. 1).

Of note, both HIV-1 and SIVagm viruses replicated to significantly higher titers in CEM-SS cells when compared with A3.01 cells (supplemental Fig. 1). However, the relative inefficient production of SIVagm from A3.01 cells relative to CEM-SS cells is not because of a SIVagm-specific restriction in these cells because HIV-1 NL4-3 virus also replicated much less efficiently in these cells than in CEM-SS cells (supplemental Fig. 1). In fact, wild type SIVagm and HIV-1 NL4-3 replicated in A3.01 cells to very similar titers. These results suggest that SIVagm Vif can target hAPO3G in A3.01 cells with similar efficiency than HIV-1 Vif.

SIVagm Vif Does Not Affect Intracellular Stability and Does Not Inhibit Encapsidation of hApo3G—To investigate further the effect of SIVagm Vif on hApo3G expressed in A3.01 cells, we studied virus production and infectivity in a single cycle of replication. For that purpose, A3.01 cells were transfected by electroporation with wild type or viif-defective SIVagm virus containing plasmid DNA. Cells and virus-containing supernatants were harvested 3 days after electroporation. Virus-containing supernatants were purified and concentrated by ultracentrifugation through 20% sucrose as described under “Experimental Procedures.” Cell lysates and viral fractions were analyzed in Fig. 2 by immunoblotting using antibodies to hApo3G, viral capsid (CA), Vif, and β-tubulin. Mock-transfected cells were included as a control for each virus. As can be seen in Fig. 2A, expression of SIVagm Vif in transfected A3.01 cells had no effect on the expression of endogenous hApo3G (Fig. 2A, lane 5), whereas HIV-1 Vif caused a modest reduction of endogenous hApo3G (Fig. 2A, lane 2) when compared with the mock control or the viif-defective samples. SIVagm viruses produced from the transfected cultures contained low but clearly detectable levels of hApo3G that were virtually identical for wild type and viif-defective viruses (Fig. 2A, lanes 5 and 6). In contrast, viif-defective HIV-1 viruses contained significantly higher levels of hApo3G than wild type viruses (Fig. 2A, compare lanes 2 and 3), consistent with the notion that HIV-1 Vif prevents the packaging of hApo3G into HIV-1 virions (5, 10, 11, 13). Nevertheless, viruses produced from A3.01 cells in the absence of Vif infected LuSIV indicator cells less efficiently than wild type viruses, consistent with the semi-permissive nature of the A3.01 cells from which the viruses originated (Fig. 2B). These results demonstrate that hApo3G expressed in A3.01 cells is functional and able to slow the replication of viif-defective SIVagm and HIV-1 viruses. In addition, these data suggest that SIVagm Vif is able to over-
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come the inhibitory activity of hApo3G in A3.01 cells with similar efficiency than HIV-1 Vif.

SIVagm Vif Inhibits Hypermutation of the Viral Genome in A3.01 Cells—To characterize further the nature of the inhibition of vif-defective SIVagm in A3.01 cells, proviral DNA was isolated from the cultures shown in Fig. 1B 14 days after infection. A 345-bp LTR fragment (Fig. 3A) and a 265-bp fragment from the env V3 region (Fig. 3B) were amplified by PCR and cloned into the pGEM-T vector (Promega). Fifteen to 18 independent clones were sequenced, and their mutation rate was analyzed. As shown in Fig. 3, replication of wild type SIVagm in A3.01 cells did not result in the accumulation of a significant number of mutations during the 2-week infection period in both 3’-LTR (Fig. 3A, wt) and env V3 regions (Fig. 3B, Wt). In contrast, hypermutation was observed for vif-defective SIVagm in A3.01 cells (Fig. 3, A and B, Vif(−)). The mutation frequency ranged from 0 to 4.5% with an average mutation frequency of 0.65% (Fig. 3C); this is lower than the 4–6% mutation frequency observed for vif-defective HIV-1 in previous reports (7, 8) and may reflect the semi-permissive nature of the host cells used in our study. The majority of mutations were G to A substitutions that occurred in regions of multiple G residues, which is indicative of hApo3G activity (Fig. 3, A and B). In the more than 5000 bp sequenced, only a single mutation occurred in a sequence context preferred by APO3F (32). It is therefore unlikely that APO3F significantly contributed to the inhibition of virus replication in A3.01 cells. These results provide further evidence that SIVagm Vif can inhibit hApo3G activity in A3.01 cells.

SIVagm Vif Does Not Inhibit Packaging of hApo3G but Rescues Viral Infectivity in a Dose-dependent Manner—To characterize further the impact of hApo3G on the infectivity of SIVagm, we performed single cycle infection studies employing virus stocks produced in HeLa cells in the presence of exogenously expressed hApo3G and various amounts of Vif protein (Fig. 4, A and B). SIVagm viruses produced in the absence of Vif packaged high levels of hApo3G (Fig. 4A, lane 1) and were completely noninfectious (Fig. 4B, lanes 1), attesting to the biological activity of our hApo3G clone. We then measured the dose-dependent effects of HIV-1 (lanes 2 and 3) or SIVagm Vif (lanes 4 and 5) on the infectivity of SIVagm.

Expression of HIV-1 Vif caused a 2–3-fold reduction in intracellular hApo3G levels (Fig. 4A, lanes 2 and 3). Most interestingly, this intracellular reduction of hApo3G did not change significantly with increasing expression of Vif (Fig. 4A, compare lanes 2 and 3). Furthermore, whereas HIV-1 Vif clearly reduced the packaging of hApo3G into SIVagm virions, this effect of HIV-1 Vif was also not dose-dependent (Fig. 4A, lanes 2 and 3). Yet increased expression of HIV-1 Vif was associated with a dose-dependent increase in the infectivity of SIVagm viruses (Fig. 4B, lanes 2 and 3). Thus, the increased infectivity of SIVagm viruses in response to increasing amounts of HIV-1 Vif cannot be explained by a dose-dependent inhibition of hApo3G encapsidation and points to an additional function of Vif.

Unlike HIV-1 Vif, SIVagm Vif had no effect on hApo3G expression, and packaging of hApo3G into SIVagm particles was not inhibited even at high levels of SIVagm Vif (Fig. 4A, lanes 4 and 5). Nevertheless, SIVagm Vif inhibited hApo3G in a dose-dependent manner and restored virus infectivity to about 25% of the level observed with HIV-1 Vif (Fig. 4B, compare lanes 3 and 5). These results support the notion that SIVagm Vif inactivates APOBEC3G through a mechanism that is not dependent on the intracellular degradation or the viral exclusion of the deaminase.

To investigate if the effect of SIVagm Vif observed in Fig. 4A is a general property of this protein or is restricted to hApo3G, similar experiments were performed in the presence of agmApo3G (Fig. 4C). SIVagmVif(−) viruses were produced as before from transfected HeLa cells in the presence of agmApo3G and increasing amounts of either HIV-1 Vif (Fig. 4C, lanes 7 and 8) or SIVagm Vif (lanes 9 and 10). Cell lysates and concentrated viral pellets were subjected to immunoblot analysis to identify cell- and virus-associated CA, Vif, and agmApo3G. As expected, HIV-1 Vif had no effect on the intracellular expression and the packaging of agmApo3G (Fig. 4C, lanes 6–8). Consistent with this, HIV-1 Vif was unable to restore viral infectivity (Fig. 4D, lanes 6–8). In contrast, SIVagm Vif caused a marked reduction of cellular and virus-associated agmApo3G levels (Fig. 4C, compare lanes 6 and 9), which at low levels of SIVagm Vif was similar to that observed for HIV-1 Vif on hApo3G (compare lanes 1 and 2 with 6 and 9). Most interestingly, however, this effect of SIVagm...
Vif on agmApo3G was not dose-dependent. In fact, both the levels of cell- and virus-associated agmApo3G were partially restored at higher levels of SIVagm Vif (Fig. 4C, compare lanes 9 and 10). This phenomenon, although unexplained, was highly reproducible and was observed in multiple replicate experiments. Moreover, despite the increased levels of virus-associated agmApo3G at higher levels of SIVagm Vif, viral infectivity increased in a dose-dependent manner (Fig. 4D, compare lanes 6, 9, and 10). These results suggest that the ability of SIVagm Vif to increase viral infectivity without blocking the encapsidation of Apo3G is not restricted to hApo3G but can be observed with agmApo3G as well. The results of this experiment show that SIVagm Vif and HIV-1 Vif are most effective in targeting autologous Apo3G. However, SIVagm Vif also had significant activity toward hApo3G, whereas HIV-1 Vif was unable to overcome the inhibitory activity of agmApo3G to a significant extent.

**HIV-1 but Not SIVagm Vif Inhibits Packaging of hApo3G into Viral Particles**—To investigate more carefully the packaging of hApo3G into HIV-1 or SIVagm particles, we performed step gradient analyses of purified virus stocks (Fig. 5). We have demonstrated previously that subjecting virus to step gradient centrifugation in the presence of Triton X-100 will remove the viral envelope but will leave the viral cores intact (33). Accordingly, soluble proteins will accumulate at the top of the gradient (Fig. 5, fraction a), whereas whole virus (in untreated samples) or proteins associated with the viral cores (in detergent-treated samples) are expected to accumulate at the interphase of 20/60% sucrose (fraction c). HeLa cells were used to produce wild type or vif-defective SIVagm or NL4-3 virus stocks in the presence of hApo3G. Virus-containing supernatants were harvested 48 h after transfection and purified by pelleting through 20% sucrose as described under “Experimental Procedures.” Concentrated virus preparations were then subjected to step gradient centrifugation in the presence or absence of Triton X-100 as described under “Experimental Procedures.” As predicted, viruses pelleted in the absence of detergent (Fig. 5, top panel) accumulated at the interphase of 20 and 60% sucrose (Fig. 5, untreated, lanes 3, 6, and 12 (CA)). Virus-associated hApo3G was identified in wild type SIVagm (Fig. 5, untreated, lane 3 (CA)) as
well as in *vif*-defective SIVagm and HIV-1 virions (Fig. 5, untreated, lanes 6 and 12 (CA)). In fact, the amounts of hApo3G associated with wild type and *vif*-defective SIVagm virions were indistinguishable. In contrast, only trace amounts of hApo3G were found in wild type HIV-1 virions (Fig. 5, untreated, lane 9 (hApo3G)).

Step gradient centrifugation of viruses in the presence of detergent (Fig. 5, bottom panel) removed ~60% of the viral capsid proteins, which now partitioned with the soluble fraction (Fig. 5, X-100, lanes 1, 4, 7, and 10 (CA)). The remainder of the capsid proteins remained in the viral core fraction (Fig. 5, lanes 3, 6, 9, and 12 (CA)). These results are consistent with our
Figure 5. Step gradient analysis of SIVagm and HIV-1 particles. Provirial vectors encoding wild type or vif-defective SIVagm9063 or HIV-1 NL4-3 were co-transfected into HeLa cells with pHIV-Apo3G vector DNA at a 6:1 molar ratio. Virus-containing supernatants were harvested 48 h after transfection, filtered, and concentrated by pelleting through 20% sucrose. Concentrated virus stocks were subjected to step gradient centrifugation in the absence (top panel) or presence (bottom panel) of Triton X-100. Three fractions (1.1 ml each) were collected from the top of the gradients. Fraction a represents the top of the gradient and contains soluble proteins. Fraction b represents a buffer zone of 20% sucrose that separates the soluble fraction a from the virus-containing fraction c. All samples were denatured by boiling in sample buffer and separated by gel electrophoresis (11% SDS-PAGE). Proteins were transferred to polyvinylidene difluoride membranes and subjected to immunoblotting using a vif monoclonal antibody (monoclonal antibody 319) and Jackson laboratories, and an antibody to SIV CA and Ronald Desrosiers for the SIVmac239 clone. We are grateful to Dennis Montoya for help with vector preparation and antiserum production. We also thank Vanessa Hirsch for providing SIVagm9063 and an antibody to SIV CA and Ronald Desrosiers for the SIVmac239 clone. We are grateful to Warner Greene for the Apo3G-specific rabbit polyclonal antibody and Nathaniel Landau for the SIVagm9063 vector pe-AGM-Apo3G-HA. We thank Michael Malin for the Vif monoclonal antibody (monoclonal antibody 319) and Jason

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SIVagm

HIV-1

|       | WT | Vif(-) |
|-------|----|--------|
| a     |    |        |
| b     |    |        |
| c     |    |        |

In contrast to previous report noting a 3–4-fold drop in hApo3G3 levels following expression of SIVagm Vif (17), our results do not reveal any effect of SIVagm Vif on either endogenous or exogenously expressed hApo3G and suggest that the strategies employed by HIV-1 and SIVagm Vif proteins to inactivate Apo3G3 are mechanistically distinct. These results also suggest that determining the steady-state levels of Apo3G3 in response to Vif expression may have only limited value as an assay to measure Vif function.

Our data suggest that SIVagm Vif prevents hypermutation of the SIVagm genome by hApo3G3 or agmApo3G3 without preventing the packaging of the deaminase into virions. Although there is no evidence from our step gradient analyses (Fig. 5) that SIVagm Vif affects the core association of Apo3G3, it is possible that Vif, which itself is an integral part of the viral core (33), inhibits the activity of Apo3G3 in the core through an as yet undefined mechanism. It is unclear why SIVagm replication in human PBMC is donor-dependent. However, we noted in Fig. 1A a significant donor-dependent variation of Apo3G3 levels. It is therefore possible that SIVagm Vif is not fully active against hApo3G3 and is ineffective in cells in which Apo3G3 expression levels exceed a certain threshold value. This is supported by our data shown in Fig. 4 where transiently expressed hApo3G3 at levels that likely exceed those found in A3.01 cells and other T cell lines (data not shown) is completely inactivated by HIV-1 Vif but only partially controlled by SIVagm Vif.

In conclusion, it remains to be shown how SIVagm Vif controls the debilitating effects of Apo3G3 activity in SIVagm virions. However, the fact that the mutation frequency observed for wild type virus in A3.01 cells is lower than in its vif-defective counterpart clearly demonstrates that SIVagm Vif can inhibit hApo3G3 function in the course of a spreading infection.

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